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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
24 August 2023 (24.08.2023)

WIPO | PCT

(10) International Publication Number
WO 2023/159208 A3

(51) International Patent Classification:
*C07K 14/705* (2006.01) *A61P 25/04* (2006.01)
*C07K 14/72* (2006.01)

(21) International Application Number:
PCT/US2023/062859

(22) International Filing Date:
17 February 2023 (17.02.2023)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/311,817 18 February 2022 (18.02.2022) US

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

(54) Title: COMPOSITIONS AND METHODS FOR NEUROLOGICAL DISEASES

FIG. 1A

| Name | Amino Acid Mutation(s) in SEQ ID NO: 33 | Acetylcholine 10 uM ach | 30 uM ach | 50 uM ach | 100 uM ach | 300 uM ach | 500 uM ach | 1 mM ach | 3 mM ach | 5 mM ach | 10 mM ach |
|---|---|---|---|---|---|---|---|---|---|---|---|
| CODA71 | - | 15.61 | 68.51 | 74.16 | 74.94 | 65.94 | 62.78 | 74.24 | 76.27 | 77.15 | 77.96 |
| CODA75 | SEQ ID NO: 29 | -12.88 | -18.67 | -0.29 | -14.46 | -16.31 | -17.41 | -16.46 | -12.38 | -20.88 | -8.69 |
| CODA217 | W77F | -5.86 | | | 70.06 | | | 68.71 | 73.45 | | |
| CODA109 | S170T | 82.91 | 82.53 | 82.11 | 83.68 | | | 69.89 | 78.12 | | |
| CODA236 | R101F | -18.24 | | | 60 | | | 66.07 | 71.38 | | |
| CODA237 | R101M | -16.79 | | | -18.63 | | | 46.28 | 69.31 | | |
| CODA238 | R101W | -16.58 | | | 75.18 | | | 78.99 | 75.74 | | |
| CODA282 | Y115D | 6.81 | | | 1.19 | | | -14.05 | 2.37 | | |
| CODA283 | Y115E_N379K | -6.16 | | | -11.12 | | | -2.74 | 53.61 | | |
| CODA325 | L131G | 5.66 | | | 74.07 | | | 62.65 | 69.49 | | |
| CODA326 | L131A | 7.43 | | | 73.57 | | | 71.8 | 74.49 | | |
| CODA330 | L131F | 11.07 | | | 43.34 | | | 77.52 | 74.89 | | |
| CODA333 | L131S | -10.11 | | | -10.05 | | | 32.11 | 57.37 | | |
| CODA334 | L131Q | -2.63 | 30.06 | | 71.27 | | | 79.66 | 74.9 | | |
| CODA335 | L131T | -9.13 | | | -9.29 | | | 0.57 | 35.37 | | |
| CODA339 | L131D | 4.56 | | | 10.19 | | | 77.34 | 77.79 | | |
| CODA340 | L131E | 15.29 | 66.56 | | 81.31 | | | 80.77 | 77.31 | | |
| CODA342 | L131R | -2.57 | | | -3.18 | | | 78.98 | 75.75 | | |
| CODA337 | L131N | 28.32 | | | 81.8 | | | 71.22 | 73.7 | | |
| CODA945 | Q139E | -1.25 | | | 71.21 | | | 71.79 | 76.04 | | |
| CODA377 | S172D | -3.19 | | | 0.07 | | | 71.7 | 76.95 | | |
| CODA405 | Y210V | -6.31 | | | -14.48 | | | -2.82 | 1.06 | | |
| CODA407 | Y210F | -3.75 | | | -1.6 | | | 71.65 | 79.09 | | |
| CODA409 | Y210W | -5.43 | | | 17.14 | | | 78.84 | 78.55 | | |

(57) **Abstract:** Compositions and methods are provided for modulating the activity of cells using engineered receptors, polynucleotide encoded engineered receptors, and gene therapy vectors comprising polynucleotides encoding engineered receptors. These compositions and methods find particular use in modulating the activity of neurons, for example in the treatment of disease or in the study of neuronal circuits.

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*[Continued on next page]*

TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

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

**Published:**

— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
— *with sequence listing part of description (Rule 5.2(a))*

(88) **Date of publication of the international search report:**
12 October 2023 (12.10.2023)

# COMPOSITIONS AND METHODS FOR NEUROLOGICAL DISEASES

## CROSS-REFERENCE TO RELATED APPLICATIONS

**[0001]** This application claims the benefit of priority to US Provisional Application No. 63/311,817 filed February 18, 2022, the content of which is incorporated herein by reference in its entirety.

## DESCRIPTION OF THE TEXT FILE SUBMITTED ELECTRONICALLY

**[0002]** The contents of the electronic sequence listing (SWCH_039_01WO_SeqList_ST26.xml; Size: 196,033 bytes; and Date of Creation: February 17, 2023) are herein incorporated by reference in its entirety.

## FIELD

**[0003]** This disclosure pertains to engineered receptors and the use of engineered receptors and small molecule ligands to modulate the activity of cells and treat diseases.

## BACKGROUND

**[0004]** Intractable neurological disease is often associated with aberrantly acting neurons. Attempts to develop therapies to treat these conditions have been hampered by a lack of tractable target proteins associated with the disease. For example, unrelieved chronic pain is a critical health problem in the US and worldwide. A report by the Institute of Medicine estimated that 116 million Americans suffer from pain that persists for weeks to years, with resulting annual costs exceeding $560 billion. There are no adequate long-term therapies for chronic pain sufferers, leading to significant cost for both society and the individual. Pain often results in disability and, even when not disabling, it has a profound effect on the quality of life. Pain treatment frequently fails even when the circumstances of care delivery are optimal, such as attentive, well-trained physicians; ready access to opioids; use of adjuvant analgesics; availability of patient-controlled analgesia; and evidence-based use of procedures like nerve blocks and IT pumps.

**[0005]** The most commonly used therapy for chronic pain is the application of opioid analgesics and nonsteroidal anti-inflammatory drugs, but these drugs can lead to addiction and may cause side effects, such as drug dependence, tolerance, respiratory depression, sedation, cognitive failure, hallucinations, and other systemic side effects. Despite the wide usage of pharmaceuticals, there is a strikingly low success rate for its effectiveness in pain relief. A large randomized study with various medications found only one out of every two or three patients

achieving at least 50% pain relief (Finnerup *et al.*, 2005). A follow-up study using the most developed pharmacological treatments found the same results, indicating that there was no improvement in the efficacy of medications for pain (Finnerup *et al.*, *Pain*, 150(3):573-81, 2010).

**[0006]** More invasive options for the treatment of pain include nerve blocks and electrical stimulation. A nerve block is a local anesthetic injection usually in the spinal cord to interrupt pain signals to the brain, the effect of which only lasts from weeks to months. Nerve blocks are not the recommended treatment option in most cases (Mailis and Taenzer, *Pain Res Manag.* 17(3):150–158, 2012). Electrical stimulation involves providing electric currents to block pain signals. Although the effect may last longer than a nerve block, complications arise with the electrical leads itself: dislocation, infection, breakage, or the battery dying. One review found that 40% of patients treated with electrical stimulation for neuropathy experienced one or more of these issues with the device (Wolter, 2014).

**[0007]** The most invasive, and least preferred, method for managing pain is complete surgical removal of the nerve or section thereof that is causing the pain. This option is only recommended when the patient has exhausted the former and other less invasive, treatments and found them ineffective. Radiofrequency nerve ablation uses heat to destroy problematic nerves and provides a longer pain relief than a nerve block. However, one study found no difference between the control and treatment groups in partial radiofrequency lesioning of the DRG for chronic lumbosacral radicular pain (Geurts *et al.*, 2003). Other surgical methods for surgically removing the pain nerves suffer from similar shortcomings and have serious side effects long-term, including sensory or motor deficits, or cause pain elsewhere.

**[0008]** Methods for treating neurological disorders should be safe, efficient and cost-effective. Gene therapy could provide non-invasive treatment options for a variety of neurological diseases, including managing pain. However, to date, gene therapy methods have not found widespread use in the treatment of neurological diseases. The present disclosure addresses these needs.

## BRIEF DESCRIPTION OF THE DRAWINGS

**[0009]** The disclosure is best understood from the following detailed description when read in conjunction with the accompanying drawings. The patent or application file contains at least one drawing executed in color. It is emphasized that, according to common practice, the various features of the drawings are not to-scale. On the contrary, the dimensions of the various

features are arbitrarily expanded or reduced for clarity. Included in the drawings are the following figures:

**[0010]** **FIG. 1A – FIG. 1J** show the heat maps of the percent quench of YFP fluorescence in the mutants of the engineered chimeric receptor SEQ ID NO: 33 that comprise the indicated amino acid substitutions following stimulation by various doses of either acetylcholine or the indicated non-native ligand. Ligand doses are written across the top of each chart. Numbers in the boxes indicate the relative amount of quench observed. Higher level of quench indicates higher level of receptor activation by the ligand at the indicated dose. Quench level is also indicated by color gradient. Dark color = more than 70% of maximal quench of YFP reporter (*i.e.*, most of the engineered receptors are activated). White = less than 10% quench (*i.e.*, few of the engineered receptors are activated). Negative values represent non-responders that have a negative quench due to a stimulation artifact. SEQ ID NO: 29 is a non-responding chimera used as a negative control. CODA283 contains an extra N379K mutation introduced during cloning, which is located outside the ligand binding domain and therefore does not affect ligand binding. **FIG. 1A** shows the YFP fluorescence quenching using acetylcholine; **FIG. 1B** shows the YFP fluorescence quenching using CNL001; **FIG. 1C** shows the YFP fluorescence quenching using TC-6683; **FIG. 1D** shows the YFP fluorescence quenching using TC-5619/Bradanicline; **FIG. 1E** shows the YFP fluorescence quenching using CNL002; **FIG. 1F** shows the YFP fluorescence quenching using ABT-126; **FIG. 1G** shows the YFP fluorescence quenching using AZD-0328; **FIG. 1H** shows the YFP fluorescence quenching using Facinicline; **FIG. 1I** shows the YFP fluorescence quenching using TC-6987; **FIG. 1J** shows the YFP fluorescence quenching using Varenicline. Abbreviations for non-native ligand names: abt: ABT-126; ach: acetylcholine; azd: AZD-0328; brd: TC-5619 (Bradanicline); fac: RG3487 (Facinicline); tc6: TC-6987; var: Varenicline.

**[0011]** **FIG. 2A – FIG. 2B** show the concentration-response curves of CR-11 (Chemogenic receptor-11, an engineered receptor comprising an amino acid sequence having the amino acid substitutions of Y115D and L131Q in SEQ ID NO: 33) expressed in HEK 293 cells to acetylcholine as well as to non-native ligands RG-3487 (SA-2, Synthetic Agonist-2). The responses were evaluated using manual patch clamp electrophysiology. Currents were normalized to unity for the maximal response. The continuous line through the data points is the best fit obtained with the Hill equation, and $EC_{50}$s for each ligand are estimated from the concentration-response curves. **FIG. 2A** shows the concentration-response curves of wild type and CR-11 receptors to acetylcholine. **FIG. 2B** shows the concentration-response curves of wild type and CR-11 receptors to RG-3487 (SA-2).

**[0012]** **FIG. 3** shows exemplary chloride currents induced by RG-3487 (SA-2) in adult rat DRG neurons transduced with a Lentivirus expressing CR-11 (Chemogenic receptor-11, an engineered receptor comprising an amino acid sequence having the amino acid substitutions of Y115D and L131Q in SEQ ID NO: 33).

**[0013]** **FIG. 4A** shows the evoked action potential of transduced DRG neurons expressing CR-11 (an engineered receptor comprising an amino acid sequence having the amino acid substitutions of Y115D and L131Q in SEQ ID NO: 33) or control DRG neurons (without CR-11 expression) at different current injections (50 pA to 700 pA). The upper panel shows the evoked action potential of a control DGF neuron. The lower left panel shows the evoked action potential of a transduced DRG neuron expressing CR-11 in the presence of 3 μM RG-3487 (SA-2). The lower right panel shows the evoked action potential of a transduced DRG neuron expressing CR-11 after RG-3487 (SA-2) is washed away. **FIG. 4B** shows the rheobase value (current required to elicit action potentials) for the control DRG neurons and transduced DRG neurons expressing CR-11 in the absence or presence of indicated ligand.

**[0014]** **FIG. 5** shows the % of HA-tag positive cells that are expressing the engineered receptors, normalized to control cells expressing the amino acid sequence of SEQ ID NO: 33 ("Normalized HA %"); and the % of α-bungarotoxin positive cells that are expressing the engineered receptors, normalized to control cells expressing the amino acid sequence of SEQ ID NO: 33 ("Normalized AB %"). **FIG. 5** also shows the median fluorescent intensity (MFI) of a cell expressing the engineered receptors, normalized to control cells expressing the amino acid sequence of SEQ ID NO: 33, as evaluated using anti-HA antibodies ("Normalized HA MFI") or fluorescently labeled α-bungarotoxin conjugated to Alexa Fluor 647 ("Normalized AB MFI").

**[0015]** **FIG. 6A** shows the relative surface and total expression of the indicated engineered receptors in cultured DRG neurons or in the HEK cells. **FIG. 6B** shows the relative surface and total expression of the indicated engineered receptors in cultured hippocampal neurons.

## SUMMARY

**[0016]** The disclosure provides engineered receptors comprising a ligand binding domain derived from human α7 nicotinic acetylcholine receptor (α7-nAChR), wherein the ligand binding domain comprises an amino acid mutation at an amino acid residue corresponding to R101, Y115, L131, Q139, Y140, S172, Y210 or Y217 of SEQ ID NO: 4. In some embodiments, the ligand binding domain comprises an amino acid sequence having at

least 85% identity to amino acid residues 23-220 of SEQ ID NO: 4. In some embodiments, the ligand binding domain comprises the amino acid mutation at two or more amino acid residues selected from those corresponding to R101, Y115, L131, Q139, Y140, S172, Y210 and Y217 of SEQ ID NO: 4. In some embodiments, the ligand binding domain comprises a mutation at the amino acid residue(s) corresponding to the indicated position of SEQ ID NO: 4:

a) Y140;

b) Y115 and L131;

c) Q139 and S172;

d) Q139 and Y210;

e) Q139, S172, and Y217;

f) Y115, Q139, and S172;

g) R101, L131, Q139, and Y217;

h) Y115 and S172;

i) L141, S172, and Y210; or

j) R101, L131, and Y217.

**[0017]** In some embodiments, the mutation is amino acid substitution. In some embodiments, the ligand binding domain comprises the amino acid substitutions(s) corresponding to the indicated position of SEQ ID NO: 4:

a) Y140R;

b) Y115E and L131M;

c) Q139W and S172D;

d) Q139W and Y210W;

e) Q139W, S172D, and Y217I;

f) Y115E, Q139E, and S172D;

g) R101F, L131G, Q139L, and Y217F;

h) Y115Q, and S172D;

i) L141M, S172D, and Y210W;

j) R101F, L131N, and Y217F;

k) L131G;

l) L131M;

m) Q139E;

n) Q139L;

o) Q139W; or

p) L141M.

**[0018]** In some embodiments, the engineered receptor is a chimeric ligand gated ion channel (LGIC) receptor comprising an ion pore domain derived from a human Glycine receptor. In some embodiments, the human Glycine receptor is human Glycine receptor α1, human Glycine receptor α2, or human Glycine receptor α3. In some embodiments, the ion pore domain comprises an amino acid sequence having at least 85% identity to amino acids 255-457 of SEQ ID NO: 2, 260-452 of SEQ ID NO: 83, amino acids 259-464 of SEQ ID NO: 85, or amino acids 259-449 of SEQ ID NO: 87. In some embodiments, the ligand binding domain of the engineered receptor comprises a Cys-loop domain derived from the human Glycine receptor. In some embodiments, the Cys-loop domain comprises amino acids 166-172 of SEQ ID NO: 2. In some embodiments, the Cys-loop domain comprises amino acids 166-180 of SEQ ID NO: 2. In some embodiments, the ligand binding domain of the engineered receptor comprises a β1-2 loop domain from the human Glycine receptor α1 subunit. In some embodiments, the β1-2 loop domain comprises amino acids 81-84 of SEQ ID NO:2. In some embodiments, the engineered receptor comprises an amino acid sequence according to any one of SEQ ID NO: 89-98.

**[0019]** The disclosure provides engineered chimeric ligand gated ion channel (LGIC) comprising a ligand binding domain derived from a first LGIC and an ion pore domain derived from a second LGIC, wherein the first LGIC is the human α7 nicotinic acetylcholine receptor (α7-nAChR) and comprises an amino acid mutation at an amino acid residue corresponding to R101, Y115, L131, Q139, Y140, S172, Y210 or Y217 of SEQ ID NO: 4.

**[0020]** In some embodiments, the ligand binding domain comprises a mutation at the amino acid residue(s) corresponding to the indicated position of SEQ ID NO: 4:

a) Y140;

b) Y115 and L131;

c) Q139 and S172;

d) Q139 and Y210;

e) Q139, S172, and Y217;

f) Y115, Q139, and S172;

g) R101, L131, Q139, and Y217;

h) Y115 and S172;

i) L141, S172, and Y210; or

j) R101, L131, and Y217.

**[0021]** In some embodiments, the ligand binding domain comprises the amino acid substitutions(s) corresponding to the indicated position of SEQ ID NO: 4:

a) Y140R;

b) Y115E and L131M;

c) Q139W and S172D;

d) Q139W and Y210W;

e) Q139W, S172D, and Y217I;

f) Y115E, Q139E, and S172D;

g) R101F, L131G, Q139L, and Y217F;

h) Y115Q, and S172D;

i) L141M, S172D, and Y210W;

j) R101F, L131N, and Y217F;

k) L131G;

l) L131M;

m) Q139E;

n) Q139L;

o) Q139W; or

p) L141M.

**[0022]** In some embodiments, the second LGIC is a human Glycine receptor. In some embodiments, the human Glycine receptor is human Glycine receptor α1. In some embodiments, the engineered chimeric LGIC comprises a polypeptide sequence having at least 85% sequence identity to SEQ ID NO: 33.

**[0023]** In some embodiments, the potency of the engineered receptor to acetylcholine is lower than the potency of the human α7 nicotinic acetylcholine receptor (α7-nAChR), or a control receptor, to acetylcholine. In some embodiments, the potency of the engineered receptor to acetylcholine is at least 2-fold lower than the potency of the human α7 nicotinic acetylcholine receptor (α7-nAChR), or a control receptor, to acetylcholine. In some embodiments, the potency of the engineered receptor to a non-native ligand is about the same as the potency of the human α7 nicotinic acetylcholine receptor (α7-nAChR), or a control receptor, to the non-native ligand. In some embodiments, the potency of the engineered receptor to a non-native ligand is higher than the potency of the human α7 nicotinic

acetylcholine receptor (α7-nAChR), or a control receptor, to the non-native ligand. In some embodiments, the potency of the engineered receptor to the non-native ligand is at least 2-fold higher than the potency of the human α7 nicotinic acetylcholine receptor (α7-nAChR), or a control receptor, to the non-native ligand. In some embodiments, the potency of the engineered receptor to a ligand is determined by the EC50 of the receptor for the ligand according to the YFP fluorescence quenching assay using Lenti-X 293T cells.

**[0024]** In some embodiments, the efficacy of the engineered receptor in the presence of a non-native ligand is higher than the efficacy the human α7 nicotinic acetylcholine receptor (α7-nAChR), or a control receptor, in presence of the non-native ligand. In some embodiments, the efficacy of the engineered receptor in the presence of a non-native ligand is at least 2-fold higher than the efficacy the human α7 nicotinic acetylcholine receptor (α7-nAChR), or a control receptor, in presence of the non-native ligand. In some embodiments, determining the efficacy comprises determining the amount of current passed through the engineered receptor in vitro in the presence of the non-native ligand.

**[0025]** In some embodiments, the non-native ligand is selected from the group consisting of AZD-0328, TC-6987, ABT-126, CNL002, TC-5619, CNL001, TC-6683, Varenicline, and Facinicline/RG3487. In some embodiments, the non-native ligand is ABT-126. In some embodiments, the non-native ligand is varenicline.

**[0026]** The disclosure provides polynucleotides encoding an engineered receptor of the disclosure. In some embodiments, the polynucleotide encodes an engineered receptor comprising an amino acid sequence of any one of SEQ ID NOs: 89-98. In some embodiments, the polynucleotide comprises a promoter operably linked to the nucleic acid encoding the engineered receptor. In some embodiments, the promoter is a regulatable promoter. In some embodiments, the regulatable promoter is active in an excitable cell. In some embodiments, the excitable cell is a neuron or a myocyte. In some embodiments, the excitable cell is a neuron.

**[0027]** The disclosure provides vectors comprising any one of the polynucleotides disclosed herein. In some embodiments, the vector is a plasmid, or a viral vector. In some embodiments, the vector is a viral vector selected from the group consisting of an adenoviral vector, a retroviral vector, an adeno-associated viral (AAV) vector, and a herpes simplex-1 viral vector (HSV-1). In some embodiments, the viral vector is an AVV vector, and wherein the AAV vector is AAV5 or a variant thereof, AAV6 or a variant thereof or AAV9 or a variant thereof. In some embodiments, the capsid protein of the AAV9 vector comprises a T492V mutation according to SEQ ID NO: 81.

**[0028]** The disclosure provides compositions comprising any one of the engineered receptors disclosed herein, any one of the polynucleotides disclosed herein, or any one of the vectors disclosed herein. The disclosure further provides pharmaceutical compositions comprising any one of the engineered receptors disclosed herein, any one of the polynucleotides disclosed herein, or any one of the vectors disclosed herein; and a pharmaceutically acceptable carrier.

**[0029]** The disclosure provides methods of expressing an engineered receptor in a neuron, comprising contacting the neuron with any one of the polynucleotides disclosed herein, any one of the vectors disclosed herein, any one of the compositions disclosed herein, or any one of the pharmaceutical compositions disclosed herein. In some embodiments, the neuron is a neuron of the peripheral nervous system. In some embodiments, the neuron is a neuron of the central nervous system. In some embodiments, the neuron is a nociceptive neuron. In some embodiments, the neuron is a non-nociceptive neuron. In some embodiments, the neuron is a hippocampal neuron, a dorsal root ganglion (DRG) neuron, a trigeminal ganglion (TG) neuron, a motor neuron, an excitatory neuron, an inhibitory neuron, or a sensory neuron. In some embodiments, the neuron is an Aδ afferent fiber, a C fiber or an Aβ afferent fiber. In some embodiments, the neuron is Aβ afferent fiber. In some embodiments, the Aβ afferent fiber is an injured Aβ afferent fiber. In some embodiments, the Aβ afferent fiber is an uninjured Aβ afferent fiber. In some embodiments, wherein the neuron expresses neurofilament 200 (NF200), piezo 2, and TLR-5. In some embodiments, the neuron does not express TrpV1, prostatic acid phosphatase, NaV1.1.

**[0030]** In some embodiments, the contacting step is performed *in vitro*, *ex vivo*, or *in vivo*. In some embodiments, the contacting step is performed *in vivo* in a subject. In some embodiments, the contacting step comprises administering the polynucleotide, the vector, the composition, or the pharmaceutical composition to the subject. In some embodiments, the contacting step is performed *in vitro* or *ex vivo*. In some embodiments, the contacting step comprises lipofection, nanoparticle delivery, particle bombardment, electroporation, sonication, or microinjection. In some embodiments, the engineered receptor is capable of localizing to the cell surface of the neuron.

**[0031]** The disclosure provides methods of inhibiting the activity of a neuron, comprising (a) contacting the neuron with any one of the engineered receptors disclosed herein, any one of the polynucleotides disclosed herein, any one of the vectors disclosed herein, any one of the compositions disclosed herein, or any one of the pharmaceutical compositions disclosed herein, and (b) contacting the neuron with a non-native ligand of the engineered

receptor. In some embodiments, the neuron is a neuron of the peripheral nervous system. In some embodiments, the neuron is a neuron of the central nervous system. In some embodiments, the neuron is a nociceptive neuron. In some embodiments, the neuron is a non-nociceptive neuron. In some embodiments, the neuron is a dorsal root ganglion (DRG) neuron, a trigeminal ganglion (TG) neuron, a motor neuron, an excitatory neuron, an inhibitory neuron, or a sensory neuron. In some embodiments, the neuron is an Aδ afferent fiber, a C fiber or an Aβ afferent fiber. In some embodiments, the neuron is Aβ afferent fiber. In some embodiments, the Aβ afferent fiber is an injured Aβ afferent fiber. In some embodiments, the Aβ afferent fiber is an uninjured Aβ afferent fiber. In some embodiments, the neuron expresses neurofilament 200 (NF200), piezo 2, and TLR-5. In some embodiments, the neuron does not express TrpV1, prostatic acid phosphatase, NaV1.1.

**[0032]** In some embodiments, the contacting step (a) is performed *in vitro*, *ex vivo*, or *in vivo*. In some embodiments, the contacting step (b) is performed *in vitro*, *ex vivo*, or *in vivo*. In some embodiments, the contacting steps (a) and/or (b) are performed *in vivo* in a subject. In some embodiments, the contacting step (a) comprises administering the engineered receptor, the polynucleotide, the vector, or the pharmaceutical composition to the subject; and/or the contacting step (b) comprises administering the non-native ligand to the subject. In some embodiments, the contacting step (a) and/or (b) comprises lipofection, nanoparticle delivery, particle bombardment, electroporation, sonication, or microinjection. In some embodiments, the engineered receptor is capable of localizing to the cell surface of the neuron.

**[0033]** The disclosure provides methods of treating and/or delaying the onset of a neurological disorder in a subject, in need thereof, comprising: administering to the subject, a therapeutically effective amount of any one of the engineered receptors disclosed herein, any one of the polynucleotides disclosed herein, any one of the vectors disclosed herein, any one of the compositions disclosed herein, or any one of the pharmaceutical compositions disclosed herein, and administering to the subject a non-native ligand of the engineered receptor. In some embodiments, the subject is administered the non-native ligand after step (a). In some embodiments, the subject is administered the non-native ligand concurrently with step (a).

**[0034]** In some embodiments, the neurological disorder is a seizure disorder, a movement disorder, an eating disorder, a spinal cord injury, neurogenic bladder, allodynia, a spasticity disorder, pruritus, Alzheimer's disease, Parkinson's disease, post-traumatic stress disorder (PTSD), gastroesophageal reflux disease (GERD), addiction, anxiety, depression, memory loss, dementia, sleep apnea, stroke, narcolepsy, urinary incontinence, essential tremor, trigeminal neuralgia, burning mouth syndrome, or atrial fibrillation. In some embodiments, the

neurological disorder is allodynia. In some embodiments, the non-native ligand is selected from the group consisting of AZD-0328, ABT-126, TC6987, CNL002, TC-5619, CNL001, TC-6683, Varenicline, and Facinicline/RG3487.

**[0035]** In some embodiments, the non-native ligand is administered orally, subcutaneously, topically, or intravenously. In some embodiments, the non-native ligand is administered orally. In some embodiments, the engineered receptor, the polynucleotide, the vector, the composition, or the pharmaceutical composition is administered subcutaneously, orally, intrathecally, topically, intravenously, intraganglioncally, intraneurally, intracranially, intraspinally, or to the cisterna magna. In some embodiments, the engineered receptor, the polynucleotide, the vector, the composition, or the pharmaceutical composition is administered by transforaminal injection or intrathecally. In some embodiments, the subject suffers from trigeminal neuralgia, and wherein the engineered receptor, the polynucleotide, the vector, the composition, or the pharmaceutical composition is administered to the trigeminal ganglion (TG) of the subject. In some embodiments, the subject suffers from neuropathic pain, and wherein the engineered receptor, the polynucleotide, the vector, the composition, or the pharmaceutical composition is administered to the dorsal root ganglion (DRG) of the subject. In some embodiments, the subject is a human.

**[0036]** In some embodiments, the therapeutically effectively amount diminishes the severity of a sign and/or or a symptom of the neurological disorder. In some embodiments, the therapeutically effectively amount delays the onset of a sign and/or or a symptom of the neurological disorder. In some embodiments, the therapeutically effectively amount eliminates a sign and/or or a symptom of the neurological disorder. In some embodiments, the sign of the neurological disorder is nerve damage, nerve atrophy, and/or seizure. In some embodiments, the nerve damage is peripheral nerve damage. In some embodiments, the symptom of the neurological disorder is pain.

**[0037]** The disclosure provides methods of treating and/or delaying the onset of pain in a subject, in need thereof, comprising: administering to the subject, a therapeutically effective amount of any one of the engineered receptors disclosed herein, any one of the polynucleotides disclosed herein, any one of the vectors disclosed herein, any one of the compositions disclosed herein, or any one of the pharmaceutical compositions disclosed herein, and administering to the subject a non-native ligand of the engineered receptor. In some embodiments, the subject is administered the non-native ligand after step (a). In some embodiments, the subject is administered the non-native ligand concurrently with step (a). In some embodiments, the non-native ligand is selected from the group consisting of AZD-0328,

ABT-126, TC6987, CNL002, TC-5619, CNL001, TC-6683, Varenicline, and Facinicline/RG3487.

**[0038]** In some embodiments, the non-native ligand is administered orally, subcutaneously, topically, or intravenously. In some embodiments, the non-native ligand is administered orally. In some embodiments, the engineered receptor, the polynucleotide, the vector, the composition, or the pharmaceutical composition is administered subcutaneously, orally, intrathecally, topically, intravenously, intraganglioncally, intraneurally, intracranially, intraspinally, or to the cisterna magna. In some embodiments, the engineered receptor, the polynucleotide, the vector, the composition, or the pharmaceutical composition is administered by transforaminal injection or intrathecally.

**[0039]** In some embodiments, the subject suffers from trigeminal neuralgia, and wherein the engineered receptor, the polynucleotide, the vector, the composition, or the pharmaceutical composition is administered to the trigeminal ganglion (TG) of the subject. In some embodiments, the subject suffers from neuropathic pain, and wherein the engineered receptor, the polynucleotide, the vector, the composition, or the pharmaceutical composition is administered to the dorsal root ganglion (DRG) of the subject.

**[0040]** In some embodiments, the subject is a human. In some embodiments, the pain is neuropathic pain. In some embodiments, the pain is associated with, caused by, or resulting from chemotherapy. In some embodiments, the pain is associated with, caused by, or resulting from trauma. In some embodiments, the subject suffers from allodynia. In some embodiments, the pain manifests after a medical procedure. In some embodiments, the pain is associated with, is caused by, or resulting from childbirth or Caesarean section. In some embodiments, the pain is associated with, is caused by, or resulting from migraine. In some embodiments, the therapeutically effectively amount diminishes pain in the subject transiently, diminishes pain in the subject permanently, prevents the onset of pain in the subject, and/or eliminates pain in the subject. In some embodiments, steps (a) and (b) are performed before the manifestation of pain in the subject.

**[0041]** The disclosure provides kits comprising (a) a vector of the disclosure and (b) a non-native ligand of the engineered receptor encoded by the vector. The disclosure provides kits comprising (a) the engineered receptor of the disclosure, and (b) a non-native ligand of the engineered receptor. In some embodiments, the combination of engineered receptor and the non-native ligand is according to any one the combinations provided in Table 29. In some embodiments, the kit comprises a device adapted to administration of the vector.

**[0042]** The disclosure provides methods of treating focal epilepsy in a subject in need thereof, comprising administering an effective amount of the polynucleotide of the disclosure or the vector of the disclosure. In some embodiments, the polynucleotide is delivered to a hippocampal neuron in the subject, or wherein the vector transduces the hippocampal neuron in the subject. In some embodiments, the vector is an AAV9 vector comprising a capsid polypeptide comprising or consisting of an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 81. In some embodiments, the focal epilepsy is mesial temporal lobe epilepsy (mTLE). In some embodiments, the polynucleotide or the vector is administered by intracranial administration, intrathecal (spine) administration, intrathecal (cisterna magna) administration, intracerebral administration, intraventricular administration, or direct injection into the epileptic focus in hippocampus.

**[0043]** The disclosure provides methods of treating neuropathic pain in a subject in need thereof, comprising administering an effective amount of the polynucleotide of the disclosure or the vector of the disclosure. In some embodiments, the neuropathic pain is peripheral neuropathy or trigeminal neuralgia. In some embodiments, the polynucleotide is delivered to a dorsal root ganglion neuron or a trigeminal ganglion neuron in the subject, or wherein the vector transduces the dorsal root ganglion neuron or the trigeminal ganglion neuron in the subject. In some embodiments, the vector is an AAV9 vector comprising a capsid polypeptide comprising or consisting of an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 81 and a non-threonine mutation at the position corresponding to T492 of SEQ ID NO: 81. In some embodiments, the non-threonine mutation is a valine substitution. In some embodiments, the polynucleotide or the vector is administered by intrathecal (IT) or intraganglionic (IG) administration.

**[0044]** In some embodiments, the method further comprises administering a non-native ligand of the engineered receptor.

## DETAILED DESCRIPTION

### A. Overview

**[0045]** Compositions and methods are provided for modulating the activity of cells using engineered ligand gated ion channel (LGIC) receptors, polynucleotide encoded engineered LGIC receptors, and gene therapy vectors comprising polynucleotides encoding engineered LGIC receptors. These compositions and methods find particular use in modulating the activity of neurons, for example in the treatment of disease or in the study of neuronal

circuits. In addition, reagents, devices and kits thereof that find use in practicing the subject methods are provided.

**[0046]** In particular, the present disclosure provides engineered receptors that bind to and signal in response to ligands. In some embodiments, the ligand is a drug. In some embodiments, the ligand is referred to as "binding agent". In some embodiments, the engineered receptors described herein demonstrate increased affinity for a known agonist ligand. In some embodiments, the engineered receptors described herein demonstrate an affinity for an antagonist or modulator ligand and respond to the antagonist and/or modulator ligands as if they were agonist ligands. The present disclosure further provides for methods of treating neurological diseases in subjects in need thereof. The present disclosure increases the number of clinical indications that a known drug may be used for by utilizing engineered receptors that respond to a known drug in a manner that is distinct from the wild-type endogenous receptor.

**[0047]** Before the present methods and compositions are described, it is to be understood that this disclosure is not limited to particular method or composition described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present disclosure will be limited only by the appended claims.

**[0048]** Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limits of that range is also specifically disclosed. Each smaller range between any stated value or intervening value in a stated range and any other stated or intervening value in that stated range is encompassed within the disclosure. The upper and lower limits of these smaller ranges may independently be included or excluded in the range, and each range where either, neither or both limits are included in the smaller ranges is also encompassed within the disclosure, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the disclosure.

**[0049]** Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, some potential and preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in

connection with which the publications are cited. It is understood that the present disclosure supersedes any disclosure of an incorporated publication to the extent there is a contradiction.

**[0050]** As will be apparent to those of skill in the art upon reading this disclosure, each of the individual embodiments described and illustrated herein has discrete components and features which may be readily separated from or combined with the features of any of the other several embodiments without departing from the scope or spirit of the present disclosure. Any recited method can be carried out in the order of events recited or in any other order which is logically possible.

**[0051]** The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present disclosure is not entitled to antedate such publication by virtue of prior disclosure. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

## B. Definitions

**[0052]** As used in this specification and the appended claims, the singular forms "a," "an" and "the" include plural references unless the content clearly dictates otherwise. Thus, for example, reference to "a cell" includes a plurality of such cells and reference to "the peptide" includes reference to one or more peptides and equivalents thereof, *e.g.* polypeptides, known to those skilled in the art, and so forth.

**[0053]** As used in this specification, the term "and/or" is used in this disclosure to either "and" or "or" unless indicated otherwise.

**[0054]** Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising" refers to the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers. Further, the statement of numerical ranges throughout this specification specifically includes all integers and decimal points in between.

**[0055]** Throughout this specification, unless the context requires otherwise, the phrase "consisting essentially of" refers to a limitation of the scope of composition, method, or kit described to the specified materials or steps that do not materially affect the basic and novel characteristic(s) of the subject disclosure. For example, a ligand binding domain "consisting essentially of" a disclosed sequence has the amino acid sequence of the disclosed sequence plus or minus about 5 amino acid residues at the boundaries of the sequence, *e.g.* about 5 residues, 4 residues, 3 residues, 2 residues or about 1 residue less than the recited bounding

amino acid residue, or about 1 residue, 2 residues, 3 residues, 4 residues, or 5 residues more than the recited bounding amino acid residue.

**[0056]** Throughout this specification, unless the context requires otherwise, the phrase "consisting of" refers to the exclusion from the composition, method, or kit of any element, step, or ingredient not specified in the claim. For example, a ligand binding domain "consisting of" a disclosed sequence consists only of the disclosed amino acid sequence.

**[0057]** As used in this application, the terms "about" and "approximately" are used as equivalents. Any numerals used in this application with or without about/approximately are meant to cover any normal fluctuations appreciated by one of ordinary skill in the relevant art. In certain embodiments, the term "approximately" or "about" refers to a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

**[0058]** As used herein, the term "isolated" means material that is substantially or essentially free from components that normally accompany is as found in its native state. In some embodiments, the terms "obtained" or "derived" are used synonymously with isolated.

**[0059]** The terms "subject," "individual," and "patient" are used interchangeably herein to refer to a vertebrate, such as a mammal. The mammal may be, for example, a mouse, a rat, a rabbit, a cat, a dog, a pig, a sheep, a horse, a non-human primate (e.g., cynomolgus monkey, chimpanzee), or a human. A subject's tissues, cells, or derivatives thereof, obtained in vivo or cultured in vitro are also encompassed. A human subject may be an adult, a teenager, a child (2 years to 14 years of age), an infant (1 month to 24 months), or a neonate (up to 1 month). In some embodiments, the adults are seniors about 65 years or older, or about 60 years or older. In some embodiments, the subject is a pregnant woman or a woman intending to become pregnant.

**[0060]** The term "sample" refers to a volume and/or mass of biological material that is subjected to analysis. In some embodiments, a sample comprises a tissue sample, cell sample, a fluid sample, and the like. In some embodiments, a sample is taken from or provided by a subject (*e.g.*, a human subject). In some embodiments, a sample comprises a portion of tissue taken from any internal organ, a cancerous, pre-cancerous, or non-cancerous tumor, brain, skin, hair (including roots), eye, muscle, bone marrow, cartilage, white adipose tissue, and/or brown adipose tissue. In some embodiments, a fluid sample comprises buccal swabs, blood, cord blood, saliva, semen, urine, ascites fluid, pleural fluid, spinal fluid, pulmonary lavage, tears,

sweat, and the like. Those of ordinary skill in the art will appreciate that, in some embodiments, a "sample" is a "primary sample" in that it is obtained directly from a source (*e.g.*, a subject). In some embodiments, a "sample" is the result of processing of a primary sample, for example to remove certain potentially contaminating components, to isolate certain components, and/or to purify certain components of interest. In some embodiments, a sample is a cell or population of cells (*e.g.*, a neuronal cell). A cell sample may be derived directly from a subject (*e.g.*, a primary sample) or may be a cell line. Cell lines may include non-mammalian cells (*e.g.*, insect cells, yeast cells, and/or bacterial cells) or mammalian cells (*e.g.*, immortalized cell lines).

**[0061]** "Treating" or "treatment" as used herein refers to delivering a composition (*e.g.*, an engineered receptor and/or a ligand) to a subject and/or population of cells to affect a physiologic outcome. In particular embodiments, treatment results in an improvement (*e.g.*, reduction, amelioration, or remediation) of one or more disease symptoms. The improvement may be an observable or measurable improvement, or may be an improvement in the general feeling of well-being of the subject. Treatment of a disease can refer to a reduction in the severity of disease symptoms. In some embodiments, treatment can refer to a reduction in the severity of disease symptoms to levels comparable to those prior to disease onset. In some embodiments, treatment may refer to a short-term (*e.g.*, temporary or acute) and/or a long-term (*e.g.*, sustained or chronic) reduction in disease symptoms. In some embodiments, treatment may refer to a remission of disease symptoms. In some embodiments, treatment may refer to the prophylactic treatment of a subject at risk of developing a particular disease in order to prevent disease development. Prevention of disease development can refer to complete prevention of the disease symptoms, a delay in disease onset, a lessening of the severity of the symptoms in a subsequently developed disease, or reducing the likelihood of disease development.

**[0062]** As used herein, "management" or "controlling" refers to the use of the compositions or methods contemplated herein, to improve the quality of life for an individual suffering from a particular disease. In certain embodiments, the compositions and methods described herein provide analgesia to a subject suffering from pain.

**[0063]** A "therapeutically effective amount" is an amount of a composition required to achieve a desired therapeutic outcome. The therapeutically effective amount may vary according to factors such as, but not limited to, disease state and age, sex, and weight of the subject. Generally, a therapeutically effective amount is also one in which any toxic or detrimental effects of a composition are outweighed by the therapeutically beneficial effects.

A "therapeutically effective amount" includes an amount of a composition that is effective to treat a subject.

**[0064]** An "increase" refers to an increase in a value (*e.g.*, increased binding affinity, increased physiologic response, increased therapeutic effect, etc.) of at least 5% as compared to a reference or control level. For example, an increase may include a 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150, 200, 250, 500, 1000% or more increase. Increase also means an increase that is 1.1, 1.2, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30 or more times (*e.g.*, 500, 1000 times) higher than a reference or control level.

**[0065]** A "decrease", "reduce", "diminish" or synonyms thereof refers to a decrease in a value (*e.g.*, decreased binding affinity, decreased physiologic response, decreased therapeutic effect, decrease in pain in a subject etc.) of at least 5% as compared to a reference or control level. For example, a decrease may include a 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150, 200, 250, 500, 1000% or more decrease. Decrease also means a decrease that is 1.1, 1.2, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30 or more times (*e.g.*, 500, 1000 times) lower than a reference or control level.

**[0066]** By "maintain," or "preserve," or "maintenance," or "no change," or "no substantial change," or "no substantial decrease" refers generally to a physiologic and/or therapeutic effect that is comparable to an effect caused by either vehicle, or a control molecule/composition. A comparable response is one that is not significantly different or measurable different from the reference response

**[0067]** The terms "reference" or "control" level are used interchangeably herein and refer the value of a particular physiologic and/or therapeutic effect in a subject or sample that has not been treated with a composition described herein, or a subject or sample that has been treated with a vehicle control. In some embodiments, a reference level refers to a value of a particular physiologic and/or therapeutic effect that is measure in a subject or sample prior to the administration of a composition described herein (*e.g.*, a baseline level).

**[0068]** As used herein, "ligand" refers to a molecule that binds to another, larger molecule. In some embodiments, the ligand binds to a receptor. In some embodiments, the binding of the ligand to the receptor alters the function of the receptor – to activate or repress its function. In some embodiments, the binding of the ligand to a receptor such a ligand gated ion channel (LGIC) leads to the opening or closing of the ion channel.

**[0069]** "Receptor-ligand binding" and "ligand binding" are used interchangeably herein and refer to the physical interaction between a receptor (*e.g.*, a LGIC) and a ligand. The term "ligand" as used herein may refer to an endogenous or naturally occurring ligand. For

example, in some embodiments, a ligand refers to a neurotransmitter (*e.g.*, λ-aminobutyric acid (GABA), acetylcholine, serotonin, and others) and signaling intermediate (*e.g.*, phosphatidylinositol 4,5-bisphosphate ($PIP_2$)), amino acids (*e.g.*, glycine), or nucleotides (*e.g.*, ATP). In some embodiments, a ligand may refer to a non-native, i.e. synthetic or non-naturally occurring, ligand. For example, in some embodiments, a ligand refers to a small molecule. Ligand binding can be measured by a variety of methods known in the art (*e.g.*, detection of association with a radioactively labeled ligand).

**[0070]** "Binding affinity" generally refers to the strength of the sum total of noncovalent interactions between a single binding site of a receptor and a ligand. Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (*e.g.,* receptor and ligand). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant ($K_d$). Affinity can be measured by common methods known in the art, including those described herein.

**[0071]** The terms "specific binding affinity" or "specific binding" are used interchangeably throughout the specification and claims and refer to binding which occurs between a paired species of molecules, *e.g.,* receptor and ligand. When the interaction of the two species produces a non-covalently bound complex, the binding which occurs is typically electrostatic, hydrogen-bonding, or the result of lipophilic interactions. In various embodiments, the specific binding between one or more species is direct. In one embodiment, the affinity of specific binding is about 2 times greater than background binding (non-specific binding), about 5 times greater than background binding, about 10 times greater than background binding, about 20 times greater than background binding, about 50 times greater than background binding, about 100 times greater than background binding, or about 1000 times greater than background binding or more.

**[0072]** "Signaling" refers to the generation of a biochemical or physiological response as a result of ligand binding to a receptor.

**[0073]** The term "wild type" or "native" is a term of the art understood by skilled persons and means the typical form of an organism, strain, gene, protein, or characteristic as it occurs in nature as distinguished from mutant or variant forms. For example, a wild type protein is the typical form of that protein as it occurs in nature.

**[0074]** The terms "non-native", "variant", and "mutant" are used interchangeably throughout the specification and the claims to refer to a mutant of a native, or wild type,

composition, for example a variant polypeptide having less than 100% sequence identity with the native, or wild type, sequence.

**[0075]** "Amino acid modifications" or "amino acid mutations" may be amino acid substitutions, amino acid deletions and/or amino acid insertions. Amino acid substitutions may be conservative amino acid substitutions or non-conservative amino acid substitutions. A conservative replacement (also called a conservative mutation, a conservative substitution or a conservative variation) is an amino acid replacement in a protein that changes a given amino acid to a different amino acid with similar biochemical properties (e.g. charge, hydrophobicity and size). As used herein, "conservative variations" refer to the replacement of an amino acid residue by another, biologically similar residue. Examples of conservative variations include the substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another; or the substitution of one polar residue for another, such as the substitution of arginine for lysine, glutamic for aspartic acids, or glutamine for asparagine, and the like. Other illustrative examples of conservative substitutions include the changes of: alanine to serine; arginine to lysine; asparagine to glutamine or histidine; aspartate to glutamate; cysteine to serine; glutamine to asparagine; glutamate to aspartate; glycine to praline; histidine to asparagine or glutamine; isoleucine to leucine or valine; leucine to valine or isoleucine; lysine to arginine, glutamine, or glutamate; methionine to leucine or isoleucine; phenylalanine to tyrosine, leucine or methionine; serine to threonine; threonine to serine; tryptophan to tyrosine; tyrosine to tryptophan or phenylalanine; valine to isoleucine or leucine, and the like.

**[0076]** The term "parental" or "starter" are used interchangeably throughout the specification and claims to refer to an initial composition, or protein that is mutated, modified, or derivatized, to create an engineered composition having novel properties. In some embodiments, the parental protein is a chimeric protein.

**[0077]** The term "engineered" is used throughout the specification and claims to refer to a non-naturally occurring composition, or protein having properties that are distinct from the parental composition, or protein from which it was derivatized.

**[0078]** In general, "sequence identity" or "sequence homology" refers to the nucleotide-to-nucleotide or amino acid-to-amino acid correspondence of two polynucleotides or polypeptide sequences, respectively. Typically, techniques for determining sequence identity include determining the nucleotide sequence of a polynucleotide and/or determining the amino acid sequence encoded thereby, and comparing these sequences to a second nucleotide or amino acid sequence. Two or more sequences (polynucleotide or amino acid) can be compared by determining their "percent identity." The percent identity of two sequences,

whether nucleic acid or amino acid sequences, is the number of exact matches between two aligned sequences divided by the length of the shorter sequences and multiplied by 100. Percent identity may also be determined, for example, by comparing sequence information using the advanced BLAST computer program, including version 2.2.9, available from the National Institutes of Health. The BLAST program is based on the alignment method of Karlin and Altschul, Proc. Natl. Acad. Sci. USA 87:2264-2268 (1990) and as discussed in Altschul, et al., J. Mol. Biol. 215:403-410 (1990); Karlin And Altschul, Proc. Natl. Acad. Sci. USA 90:5873-5877 (1993); and Altschul et al., Nucleic Acids Res. 25:3389-3402 (1997). Briefly, the BLAST program defines identity as the number of identical aligned symbols (generally nucleotides or amino acids), divided by the total number of symbols in the shorter of the two sequences. The program may be used to determine percent identity over the entire length of the proteins being compared. Default parameters are provided to optimize searches with short query sequences in, for example, with the blastp program. The program also allows use of an SEG filter to mask-off segments of the query sequences as determined by the SEG program of Wootton and Federhen, Computers and Chemistry 17:149-163 (1993). Ranges of desired degrees of sequence identity are approximately 80% to 100% and intervening integer values. Typically, the percent identities between a disclosed sequence and a claimed sequence are at least 80%, at least 85%, at least 90%, at least 95%, or at least 98%.

**[0079]** As used herein, “substantially identical” refers to having a sequence identity that is 85% or more, for example 90% or more, *e.g.* 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9%, or 100%, wherein the activity of the composition is unaltered by the modifications in the sequence that result in the difference in sequence identity.

**[0080]** As used herein in relation to the position of an amino acid or a nucleotide, the terms “corresponding to” or “correspond to" refer to an amino acid in a first polypeptide sequence that aligns with a given amino acid in a reference polypeptide sequence when the first polypeptide and reference polypeptide sequences are aligned, or a nucleotide in a first polynucleotide sequence that aligns with a given nucleotide in a reference polynucleotide sequence when the first polynucleotide and reference polynucleotide sequences are aligned. Alignment is performed by one of skill in the art using software designed for this purpose, for example, BLAST program version 2.2.9 with the default parameters for that version.

**[0081]** As used herein, the term "promoter" refers to one or more nucleic acid control sequences that direct transcription of an operably linked nucleic acid. Promoters may include nucleic acid sequences near the start site of transcription, such as a TATA element. Promoters may also include cis-acting polynucleotide sequences that can be bound by transcription

factors. A "constitutive" promoter is a promoter that is active under most environmental and developmental conditions. An "inducible" promoter is a promoter that is active under environmental or developmental regulation. The term "operably linked" refers to a functional linkage between a nucleic acid expression control sequence (such as a promoter, or array of transcription factor binding sites) and a second nucleic acid sequence, wherein the expression control sequence directs transcription of the nucleic acid corresponding to the second sequence.

**[0082]** As used herein, the terms “virus vector,” “viral vector,” or “gene delivery vector” refer to a virus particle that functions as a nucleic acid delivery vehicle, and which comprises a nucleic acid (e.g., an AAV expression cassette) packaged within a virion. Exemplary virus vectors of the disclosure include adenovirus vectors, adeno-associated virus vectors (AAVs), lentivirus vectors, and retrovirus vectors.

**[0083]** As used herein, “neuronal activity”, “activity of a neuron”, “neuronal firing” and variations and synonyms thereof, refer to the electrical activity resulting from the stimulation or excitation of a neuron. In some embodiments, neuronal activity is measured using automated or manual patch clamp techniques. In some embodiments, determining the activity of a neuron comprises determining the excitatory postsynaptic potential (EPSP), inhibitory postsynaptic potential (IPSP), and/or action potential of the neuron. In some embodiments, the level of activity of a neuron depends on, or is affected by, the excitatory postsynaptic potential (EPSP), inhibitory postsynaptic potential (IPSP), and/or action potential.

**[0084]** As used herein, a “neurological disease” or “neurological disorder” refers to a disease or disorder of the nervous system. In some embodiments, the neurological disease is associated with, caused by, or results from structural, biochemical, and/or electrical abnormalities in the brain, spinal cord, a nerve or any component of the nervous system.

**[0085]** As used herein, a “sign” of a disease refers to a physical or mental feature which is regarded as indicating a condition of disease. In some embodiments, a sign is an objective indication of the disease. In some embodiments, a sign is evaluated, examined, observed or measured objectively by a person other than the patient, such as a doctor.

**[0086]** As used herein, a “symptom” of a disease refers to a physical or mental feature which is regarded as indicating a condition of disease, particularly such a feature that is apparent to the patient. In some embodiments, the symptom is subjectively evaluated by the patient. For example, in some embodiments, the symptom is pain.

**[0087]** As used herein, “potency” refers to the ability of a receptor described herein to respond to a particular ligand. An increase in potency therefore refers to an increase in the responsiveness of the receptor for a particular ligand. A decrease in potency therefore refers to

a decrease in the responsiveness of the receptor for a particular ligand. The potency of a receptor is generally determined herein by the half maximal effective concentration (EC50) for a particular ligand of a particular receptor. The EC50 refers to the concentration of the ligand which induces a response halfway between the baseline and maximum after a specific exposure time. In some embodiments, the response is the opening or closing of the ion channel in the receptor.

**[0088]** As used herein, "substantially retaining the potency" for a ligand refers to an engineered receptor having an EC50 for a particular ligand that is unchanged, or changed by and increase or decrease of less than 2-fold, compared to a parental or control receptor..

**[0089]** As used herein, "efficacy" of a receptor in relation to a ligand refers to a measure of the activity of the receptor in the presence of a ligand. In some embodiments, the efficacy refers to the amount of current passed through the receptor under specific conditions, such as in the presence of a specific concentration of the ligand. In some embodiments, determining the efficacy comprises determining the amount of current passed through the receptor, and/or the rheobase of the receptor.

### C. Engineered Receptors

**[0090]** The present disclosure is directed to engineered receptors, engineered receptor mutants, and methods for their use. The term "receptor" as used herein refers to any protein that is situated on the surface of a cell and that can mediate signaling to and/or from the cell. The term "engineered receptor" is used herein to refer to a receptor that has been experimentally altered such that it is physically and/or functionally distinct from a corresponding parental receptor. In some embodiments, the parental receptor is a wild-type receptor. The term "wild-type receptor" is used herein to refer to a receptor having a polypeptide sequence that is identical to the polypeptide sequence of a protein found in nature. Wild-type receptors include receptors that naturally occur in humans as well as orthologs that naturally occur in other eukaryotes, *e.g.* protist, fungi, plants or animals, for example yeast, insects, nematodes, sponge, mammals, non-mammalian vertebrates. In some embodiments, the parental receptor is a non-native receptor; that is, it is a receptor that does not occur in nature, for example, a receptor that is engineered from a wild type receptor. For example, a parental receptor may be an engineered receptor comprising one or more subunits from one wild-type receptor with one or more subunits from a second wild-type receptor. The resulting proteins are therefore comprised of subunits from two or more wild-type receptors. Therefore, in some

embodiments, the parental receptor is a chimeric receptor. Engineered receptors of the present disclosure include, for example, parental receptor mutants, and switch receptors.

**[0091]** In some aspects, an engineered receptor of the present disclosure comprises at least one amino acid mutation relative to the corresponding parental receptor, *e.g.* one or more mutations in one or more domains of a wild-type receptor. In some embodiments, the mutation is an amino acid substitution. In some embodiments, the engineered receptor shares a sequence identity of about 99%, about 98%, about 95%, about 90%, about 85%, about 80%, about 70%, about 60%, about 50%, or less with the corresponding parental receptor, inclusive of all values and subranges that lie therebetween. In some embodiments, the parental receptor mutant has a sequence identity of 85% or more with the corresponding parental receptor, *e.g.* 90% or more or 95% or more, for example, about 96%, about 97%, about 98% or about 99% identity with the corresponding parental receptor, inclusive of all values and subranges that lie therebetween. In some embodiments, an engineered receptor (*e.g.*, a parental receptor mutant) is generated by error prone PCR.

**[0092]** In some embodiments, the ligand binding domain (LBD) of the engineered receptor of the disclosure comprises at least one amino acid mutation relative to the corresponding ligand binding domain of the parental receptor, e.g. one or more mutations in the ligand binding domain of a wild-type receptor. In some embodiments, the mutation is an amino acid substitution. In some embodiments, the ligand binding domain of the engineered receptor has a sequence identity of 85% or more with the corresponding ligand binding domain of the parental receptor, e.g. 90% or more or 95% or more, for example, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% identity, or 100% identity with the corresponding ligand binding domain of the parental receptor, inclusive of all values and subranges that lie therebetween. In some embodiments, the ligand binding domain of the engineered receptor shares a sequence identity of at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity with the corresponding ligand binding domain of the parental receptor, inclusive of all values and subranges that lie therebetween.

**[0093]** In some embodiments, the ion pore domain (IPD) of the engineered receptor of the disclosure comprises at least one amino acid mutation relative to the corresponding ion pore domain of the parental receptor, e.g. one or more mutations in the ion pore domain of a wild-type receptor. In some embodiments, the mutation is an amino acid substitution. In some embodiments, the ion pore domain of the engineered receptor has a sequence identity of 85% or more with the corresponding ion pore domain of the parental receptor, e.g. 90% or more or

95% or more, for example, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% identity, or 100% identity with the corresponding ion pore domain of the parental receptor, inclusive of all values and subranges that lie therebetween. In some embodiments, the ion pore domain of the engineered receptor shares a sequence identity of at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity with the corresponding ion pore domain of the parental receptor, inclusive of all values and subranges that lie therebetween.

**[0094]** In some embodiments, the amino acid mutation is a loss-of-function amino acid mutation relative to a corresponding parental receptor. "Loss-of-function" amino acid mutations refer to one or more mutations that reduce, substantially decrease, or abolish the function of the engineered receptor relative to the parental receptor, for example by reducing the binding of an endogenous ligand to an engineered receptor relative to the binding of endogenous ligand to the parental receptor, or by reducing the activity of signaling pathway(s) downstream of the engineered receptor that are typically activated in response to the binding of a ligand to the corresponding parental receptor. In some embodiments, the mutation is an amino acid substitution.

**[0095]** In some embodiments, the amino acid mutation is a gain-of-function amino acid mutation relative to a corresponding parental receptor. "Gain-of-function" amino acid mutations refer to one or more mutations that modify the function of the engineered receptor relative to the parental receptor, for example by altering or enhancing the affinity of an engineered receptor for a ligand relative to the binding of endogenous ligand to the parental receptor, or by altering or enhancing the activity of the signaling pathways that are activated in response to the binding of a ligand to an engineered receptor relative to the binding of the endogenous ligand to the corresponding parental receptor. In some embodiments, a gain-of-function mutation results in an increased affinity of the engineered receptor for a ligand. In particular embodiments, a gain-of-function mutation results in an increased affinity of the engineered receptor for an agonist ligand. In some embodiments, a gain-of-function mutation results in an antagonist ligand acting as an agonist ligand upon binding to the engineered receptor (*e.g.*, results in the activation of agonist signaling pathways instead of antagonist signaling pathways). In some embodiments, a gain-of-function mutation results in a modulator ligand acting as an agonist ligand upon binding to the engineered receptor. In some embodiments, the mutation is an amino acid substitution.

**[0096]** In some embodiments, the subject engineered receptor of the present disclosure, or the ligand binding domain and/or the ion pore domain thereof, comprises one or more loss-

of-function amino acid mutations and one or more gain-of-function amino acid mutations relative to a corresponding parental receptor. In some embodiments, the mutation is an amino acid substitution.

**[0097]** In some embodiments, the loss of function mutation and the gain of function mutation are at the same residue, i.e. they are the same mutation. In other embodiments, the loss of function mutation and the gain of function mutation are mutations at different amino acid residues. In some embodiments, the mutation is an amino acid substitution. In some embodiments, the subject engineered receptor (or the ligand binding domain and/or the ion pore domain thereof) comprising the loss of function mutation and/or gain of function mutation shares a sequence identity of about 99%, about 98%, about 95%, about 90%, about 85%, about 80%, about 70%, about 60%, about 50%, including all ranges and subranges therebetween, or less with the corresponding parental receptor, *e.g.* wild type receptor or non-native receptor (or the ligand binding domain and/or the ion pore domain thereof). In some embodiments, the subject engineered receptor (or the ligand binding domain and/or the ion pore domain thereof) shares a sequence identity of 85% or more with the corresponding parental receptor (or the ligand binding domain and/or the ion pore domain thereof) , for example 85%, 90%, or 95% or more sequence identity, in some instances 96%, 97%, 98% or more sequence identity, *e.g.* 99% or 99.5% or more sequence identity, inclusive of all values and subranges that lie therebetween.

**[0098]** In some aspects, engineered receptors of the present disclosure include receptors produced by the combination of one or more amino acid sequences, *e.g.* subunits, derived from one wild-type receptor with one or more amino acid sequences, *e.g.* subunits, derived from a second wild-type receptor. In other words, the engineered receptor comprises amino acid sequences that are heterologous to one another, where by "heterologous", it is meant not occurring together in nature. Such receptors are referred to herein as "chimeric receptors". In some embodiments, chimeric receptors serve as parental receptors from which an engineered receptor of the present disclosure is generated.

**[0099]** In some embodiments, a parental receptor mutant demonstrates increased affinity for an agonist ligand. In some embodiments, a ligand that functions as an antagonist or modulator when binding to a wild type receptor functions as an agonist when binding to a parental receptor mutant.

**[0100]** In some embodiments, the engineered receptor is a "ligand-gated ion channel" or LGIC. An LGIC refers to a large group of transmembrane proteins that allow passage of ions upon activation by a specific ligand. LGIC are composed of at least two domains: a ligand

binding domain and a transmembrane ion pore domain. Ligand binding to an LGIC results in activation of the LGIC and opening of the ion pore. Ligand binding causes a drastic change in the permeability of the channel to a specific ion or ions; effectively no ions can pass through the channel when it is inactive or closed but up to $10^7$ ions/second can pass through upon ligand binding. In some embodiments, LGICs respond to extracellular ligands (*e.g.*, neurotransmitters) and facilitate an influx of ions into the cytosol. In some embodiments, LGICs respond to intracellular ligands (*e.g.*, nucleotides such at ATP and signaling intermediates such as $PIP_2$) and facilitate an efflux of ions from the cytosol into the extracellular environment. Importantly, activation of LGIC results in the transport of ions across the cellular membrane (*e.g.*, $Ca^{2+}$, $Na^+$, $K^+$, $Cl^-$, etc.) and does not result in the transport of the ligand itself.

**[0101]** LGIC receptors are comprised of multiple subunits and can be either homomeric receptors or heteromeric receptors. A homomeric receptor is comprised of subunits that are all the same type. A heteromeric receptor is comprised of subunits wherein at least one subunit is different from at least one other subunit comprised within the receptor. For example, the glycine receptor is comprised of 5 subunits of which there are two types: α-subunits, of which there are four isoforms ($\alpha_1$ – $\alpha_4$) and β-subunits, of which there is a single known isoform. An exemplary homomeric GlyR is a GlyR comprised of 5 $\alpha_1$-GlyR subunits. Similarly, a homomeric $GABA_A$ receptor may be comprised of $\beta_3$-$GABA_A$ subunits, and an nAchR receptor may be comprised of $\alpha_7$-nAchR subunits. An exemplary heteromeric GlyR may be comprised of one or more α-subunits and one or more of β-subunits (*e.g.*, an $\alpha_1\beta$-GlyR). Subunits of example LGIC receptors are shown in **Table 1**.

**Table 1: LGIC Receptors and Subunits**

| Receptor | Subunits | Subunit Isoforms |
|---|---|---|
| GlyR | GLRA1 | |
| | GLRA2 | |
| | GLRA3 | |
| | GLRA4 | |
| | GLRB | |
| $5HT_3$ | 5-HT3A | |
| | 5-HT3B | |
| | 5-HT3C | |
| | 5-HT3D | |
| | 5-HT3E | |
| nAChR | α | α1 |
| | | α2 |
| | | α3 |
| | | α4 |
| | | α5 |

| Receptor | Subunits | Subunit Isoforms |
|---|---|---|
| | | α6 |
| | | α7 |
| | | α8 |
| | | α9 |
| | | α10 |
| | β | β1 |
| | | β2 |
| | | β3 |
| | | β4 |
| | γ | γ |
| | δ | δ |
| | ε | ε |
| $GABA_A$ | α | α1 |
| | | α2 |
| | | α3 |
| | | α4 |
| | | α5 |
| $GABA_A$ | α | α6 |
| | β | β1 |
| | | β2 |
| | | β3 |
| | γ | γ1 |
| | | γ2 |
| | | γ3 |
| | δ | δ |
| | ε | ε |
| | π | π |
| | ρ | ρ1 |
| | | ρ2 |
| | | ρ3 |
| P2X | P2X1 | |
| | P2X2 | |
| | P2X3 | |
| | P2X4 | |
| | P2X5 | |
| | P2X6 | |
| | P2X7 | |
| KCNQ | α | $K_v$α1 |
| | | $K_v$α2 |
| | | $K_v$α3 |
| | | $K_v$α4 |
| | | $K_v$α5 |
| | | $K_v$α6 |
| | | $K_v$α7 |
| | | $K_v$α8 |
| | | $K_v$α9 |
| | | $K_v$α10 |

| Receptor | Subunits | Subunit Isoforms |
|---|---|---|
| | | $K_V\alpha11$ |
| | | $K_V\alpha12$ |
| | β | $K_V\beta1$ |
| | | $K_V\beta2$ |
| | | $K_V\beta3$ |
| | | minK |
| | | MiRP1 |
| | | MiRP2 |
| | | MiRP3 |
| | | KCNE1-like |
| | | KCNIP1 |
| | | KCNIP2 |
| | | KCNIP3 |
| | | KCNIP4 |

**[0102]** Illustrative examples of families of LGICs suitable for use in particular embodiments include, but are not limited to Cys-loop receptors such as Glycine receptors (GlyR), serotonin receptors (*e.g.*, 5-HT3 receptors), λ-Aminobutyric Acid A (GABA-A) receptors, and Nicotinic acetylcholine receptors (nAchR); as well as Acid-sensing (proton-gated) ion channels (ASICs), Epithelial sodium channels (ENaC), Ionotropic glutamate receptors, IP3 receptor, P2X receptors, the Ryanodine receptor, and Zinc activated channels (ZAC).

**[0103]** Specific non-limiting examples of LGICs that are suitable for use with the methods described herein include: HTR3A; HTR3B; HTR3C; HTR3D; HTR3E; ASIC1; ASIC2; ASIC3; SCNN1A; SCNN1B; SCNN1D; SCNN1G; GABRA1; GABRA2; GABRA3; GABRA4; GABRA5; GABRA6; GABRB1; GABRB2; GABRB3; GABRG1; GABRG2; GABRG3; GABRD; GABRE; GABRQ; GABRP; GABRR1; GABRR2; GABRR3; GLRA1; GLRA2; GLRA3; GLRA4; GLRB; GRIA1; GRIA2; GRIA3; GRIA4; GRID1; GRID2; GRIK1; GRIK2; GRIK3; GRIK4; GRIK5; GRIN1; GRIN2A; GRIN2B; GRIN2C; GRIN2D; GRIN3A; GRIN3B; ITPR1; ITPR2; ITPR3; CHRNA1; CHRNA2; CHRNA3; CHRNA4; CHRNA5; CHRNA6; CHRNA7; CHRNA9; CHRNA10; CHRNB1; CHRNB2; CHRNB3; CHRNB4; CHRNG; CHRND; CHRNE; P2RX1; P2RX2; P2RX3; P2RX4; P2RX5; P2RX6; P2RX7; RYR1; RYR2; RYR3; and ZACN.

**[0104]** TRPV1, TRPM8 and $P2X_2$ are members of large LGIC families that share structural features as well as gating principles. For example TRPV4, similar to TRPV1, is also triggered by heat, but not by capsaicin; and $P2X_3$, is triggered by ATP, but desensitizes more rapidly than $P2X_2$. TRPV1, TRPM8 and $P2X_2$ are, therefore, non-limiting examples of LGIC suitable for use in particular embodiments.

**[0105]** In one embodiment, the engineered receptor is a TRPV1 or TRPM8 receptor or a mutein thereof. TRPV1 and TRPM8, are vanilloid and menthol receptors expressed by nociceptive neurons of the peripheral nervous system. Both channels are thought to function as non-selective, sodium- and calcium-permeable homotetramers. In addition, both channels and their principal agonists--capsaicin and cooling compounds, such as menthol, respectively--are virtually absent from the central nervous system. Capsaicin and some cooling compounds, including menthol and icilin, contain potential acceptor sites for photolabile blocking groups. Association of a photolabile blocking group with such an acceptor would result in a ligand-gated ion channel in which light acts as an indirect trigger by releasing the active ligand.

**[0106]** In one embodiment, the engineered receptor is a $P2X_2$ receptor or a mutein thereof. $P2X_2$ is an ATP-gated non-selective cation channel distinguished by its slow rate of desensitization. $P2X_2$ may be used as a selectively addressable source of depolarizing current and present a platform for the generation of engineered channel-ligand combinations that lack natural agonists altogether.

**[0107]** Non-limiting examples of sequences of wild-type LGIC receptor that find use in the generation of engineered receptors of the present disclosure include the following. In the sequences, the signal peptide is italicized, the ligand binding domain is bolded, and the ion pore domain is underlined:

**[0108]** In some embodiments, the wild-type LGIC receptor is a human alpha 1 glycine receptor (GlyRα1) (GenBank Accession No. NP_001139512.1, SEQ ID NO:2), encoded by the *GLRA1* gene (GenBank Accession No. NM_001146040.1 (SEQ ID NO:1):

```
    10        20        30        40        50        60
MYSFNTLRLY LWETIVFFSL AASKEAEAAR SAPKPMSPSD FLDKLMGRTS GYDARIRPNF
    70        80        90       100       110       120
KGPPVNVSCN IFINSFGSIA ETTMDYRVNI FLRQQWNDPR LAYNEYPDDS LDLDPSMLDS
    130       140       150       160       170       180
IWKPDLFFAN EKGAHFHEIT TDNKLLRISR NGNVLYSIRI TLTLACPMDL KNFPMDVQTC
    190       200       210       220       230       240
IMQLESFGYT MNDLIFEWQE QGAVQVADGL TLPQFILKEE KDLRYCTKHY NTGKFTCIEA
    250       260       270       280       290       300
RFHLERQMGY YLIQMYIPSL LIVILSWISF WINMDAAPAR VGLGITTVLT MTTQSSGSRA
    310       320       330       340       350       360
SLPKVSYVKA IDIWMAVCLL FVFSALLEYA AVNFVSRQHK ELLRFRRKRR HHKSPMLNLF
    370       380       390       400       410       420
QEDEAGEGRF NFSAYGMGPA CLQAKDGISV KGANNSNTTN PPPAPSKSPE EMRKLFIQRA
    430       440       450       457
KKIDKISRIG FPMAFLIFNM FYWIIYKIVR REDVHNQ
```

(SEQ ID NO: 2).

**[0109]** In some embodiments, the wild-type LGIC receptor is a human alpha 2 glycine receptor (GlyRα2) (GenBank Accession No. NP_001112357.1, SEQ ID NO: 83), encoded by the *GLRA2* gene (GenBank Accession No. NM_001118885.1, SEQ ID NO: 82):

```
    10      20      30      40      50      60
MNRQLVNILT ALFAFFLETN HFRTAFCKDH DSRSGKQPSQ TLSPSDFLDK LMGRTSGYDA
    70      80      90     100     110     120
RIRPNFKGPP VNVTCNIFIN SFGSVTETTM DYRVNIFLRQ QWNDSRLAYS EYPDDSLDLD
    130     140     150     160     170     180
PSMLDSIWKP DLFFANEKGA NFHDVTTDNK LLRISKNGKV LYSIRLTLTL SCPMDLKNFP
    190     200     210     220     230     240
MDVQTCTMQL ESFGYTMNDL IFEWLSDGPV QVAEGLTLPQ FILKEEKELG YCTKHYNTGK
    250     260     270     280     290     300
FTCIEVKFHL ERQMGYYLIQ MYIPSLLIVI LSWVSFWINM DAAPARVALG ITTVLTMTTQ
    310     320     330     340     350     360
SSGSRASLPK VSYVKAIDIW MAVCLLFVFA ALLEYAAVNF VSRQHKEFLR LRRRQKRQNK
    370     380     390     400     410     420
EEDVTRESRF NFSGYGMGHC LQVKDGTAVK ATPANPLPQP PKDGDAIKKK FVDRAKRIDT
    430     440     450  452
ISRAAFPLAF LIFNIFYWIT YKIIRHEDVH KK
```

(SEQ ID NO: 83).

**[0110]** In some embodiments, the wild-type LGIC receptor is a human alpha 3 glycine receptor (GlyRα3) isoform L (GenBank Accession No. NP_006520.2, SEQ ID NO: 85), encoded by the *GLRA3* gene (GenBank Accession No. NM_006529.3, SEQ ID NO: 84):

```
    10      20      30      40      50      60
MAHVRHFRTL VSGFYFWEAA LLLSLVATKE TDSARSRSAP MSPSDFLDKL MGRTSGYDAR
    70      80      90     100     110     120
IRPNFKGPPV NVTCNIFINS FGSIAETTMD YRVNIFLRQK WNDPRLAYSE YPDDSLDLDP
    130     140     150     160     170     180
SMLDSIWKPD LFFANEKGAN FHEVTTDNKL LRIFKNGNVL YSIRLTLTLS CPMDLKNFPM
    190     200     210     220     230     240
DVQTCIMQLE SFGYTMNDLI FEWQDEAPVQ VAEGLTLPQF LLKEEKDLRY CTKHYNTGKF
    250     260     270     280     290     300
TCIEVRFHLE RQMGYYLIQM YIPSLLIVIL SWVSFWINMD AAPARVALGI TTVLTMTTQS
    310     320     330     340     350     360
SGSRASLPKV SYVKAIDIWM AVCLLFVFSA LLEYAAVNFV SRQHKELLRF RRKRKNKTEA
    370     380     390     400     410     420
FALEKFYRFS DMDDEVRESR FSFTAYGMGP CLQAKDGMTP KGPNHPVQVM PKSPDEMRKV
    430     440     450     460   464
FIDRAKKIDT ISRACFPLAF LIFNIFYWVI YKILRHEDIH QQQD
```

(SEQ ID NO: 85).

**[0111]** In some embodiments, the wild-type LGIC receptor is a human alpha 3 glycine receptor (GlyRα3) isoform K (GenBank Accession No. NP_001036008.1, SEQ ID NO: 87), encoded by the *GLRA3* gene (GenBank Accession No. NM_001042543.3, SEQ ID NO: 86):

```
    10      20      30      40      50
MAHVRHFRTL VSGFYFWEAA LLLSLVATKE TDSARSRSAP MSPSDFLDKL
    60      70      80      90     100
```

**MGRTSGYDAR IRPNFKGPPV NVTCNIFINS FGSIAETTMD YRVNIFLRQK**
110 120 130 140 150
**WNDPRLAYSE YPDDSLDLDP SMLDSIWKPD LFFANEKGAN FHEVTTDNKL**
160 170 180 190 200
**LRIFKNGNVL YSIRLTLTLS CPMDLKNFPM DVQTCIMQLE SFGYTMNDLI**
210 220 230 240 250
**FEWQDEAPVQ VAEGLTLPQF LLKEEKDLRY CTKHYNTGKF TCIEVRFHLE**
260 270 280 290 300
**RQ**MGYYLIQM YIPSLLIVIL SWVSFWINMD AAPARVALGI TTVLTMTTQS
310 320 330 340 350
SGSRASLPKV SYVKAIDIWM AVCLLFVFSA LLEYAAVNFV SRQHKELLRF
360 370 380 390 400
RRKRKNKDDE VRESRFSFTA YGMGPCLQAK DGMTPKGPNH PVQVMPKSPD
410 420 430 440
EMRKVFIDRA KKIDTISRAC FPLAFLIFNI FYWVIYKILR HEDIHQQQD
(SEQ ID NO: 87).

**[0112]** In some embodiments, the wild-type LGIC receptor is a human nicotinic cholinergic receptor alpha 7 subunit (α7-nAchR) (GenBank Accession No. NP_000737.1, SEQ ID NO:4), encoded by the *CHRNA7* gene (GenBank Accession No. NM_000746.5 (SEQ ID NO:3):

10 20 30 40 50 60
*MRCSPGGVWL ALAASLLHVS LQ***GEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL**
70 80 90 100 110 120
**QIMDVDEKNQ VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV RFPDGQIWKP DILLYNSADE**
130 140 150 160 170 180
**RFDATFHTNV LVNSSGHCQY LPPGIFKSSC YIDVRWFPFD VQHCKLKFGS WSYGGWSLDL**
190 200 210 220 230 240
**QMQEADISGY IPNGEWDLVG IPGKRSERFY ECCKEPYPDV TFTVTMRRR**T LYYGLNLLIP
250 260 270 280 290 300
CVLISALALL VFLLPADSGE KISLGITVLL SLTVFMLLVA EIMPATSDSV PLIAQYFAST
310 320 330 340 350 360
MIIVGLSVVV TVIVLQYHHH DPDGGKMPKW TRVILLNWCA WFLRMKRPGE DKVRPACQHK
370 380 390 400 410 420
QRRCSLASVE MSAVAPPPAS NGNLLYIGFR GLDGVHCVPT PDSGVVCGRM ACSPTHDEHL
430 440 450 460 470 480
LHGGQPPEGD PDLAKILEEV RYIANRFRCQ DESEAVCSEW KFAACVVDRL CLMAFSVFTI
490 500 502
ICTIGILMSA PNFVEAVSKD FA

(SEQ ID NO:4).

**[0113]** In some embodiments, the wild-type LGIC receptor is a human 5-hydroxytryptamine receptor 3A (5HT3A, GenBank Accession No. NP_998786.2, SEQ ID NO:6), encoded by the *HTR3A* gene (GenBank Accession No. NM_213621.3, SEQ ID NO:5):

**(a)** *MLGKLAMLLWVQQALLALLLPTL***LAQGEARRSRNTTRPALLRLSDYL LTNYRKGVRPVRDWRKPTTVSIDVIVYAILNVDEKNQVLTTYIWYRQYWTDEF LQWNPEDFDNITKLSIPTDSIWVPDILINEFVDVGKSPNIPYVYIRHQGEVQNYKP**

**LQVVTACSLDIYNFPFDVQNCSLTFTSWLHTIQDINISLWRLPEKVKSDRSVFMN QGEWELLGVLPYFREFSMESSNYYAEMKFYVVIRRR**PLFYVVSLLLPSIFLMVMD IVGFYLPPNSGERVSFKITLLLGYSVFLIIVSDTLPATAIGTPLIGKAPPGSRAQSGEKPA PSHLLHVSLASALGCTGVYFVVCMALLVISLAETIFIVRLVHKQDLQQPVPAWLRHL VLERIAWLLCLREQSTSQRPPATSQATKTDDCSAMGNHCSHMGGPQDFEKSPRDRC SPPPPPREASLAVCGLLQELSSIRQFLEKRDEIREVARDWLRVGSVLDKLLFHIYLLAV LAYSITLVMLWSIWQYA (SEQ ID NO:6).

**[0114]** In some embodiments, the wild-type LGIC receptor is a human 5-hydroxytryptamine receptor 3B (5HT3B GenBank Accession No. NP_006019.1, SEQ ID NO:57), encoded by the *HTR3B* gene (GenBank Accession No. NM_006028.4, SEQ ID NO:56):

**(a)** *MLSSVMAPLWACILVAAGILA***TDTHHPQDSALYHLSKQLLQKYHKEV RPVYNWTKATTVYLDLFVHAILDVDAENQILKTSVWYQEVWNDEFLSWNSSMF DEIREISLPLSAIWAPDIIINEFVDIERYPDLPYVYVNSSGTIENYKPIQVVSACSLE TYAFPFDVQNCSLTFKSILHTVEDVDLAFLRSPEDIQHDKKAFLNDSEWELLSVS STYSILQSSAGGFAQIQFNVVMRRHP**LVYVVSLLIPSIFLMLVDLGSFYLPPNCRARI VFKTSVLVGYTVFRVNMSNQVPRSVGSTPLIGHFFTICMAFLVLSLAKSIVLVKFLHD EQRGGQEQPFLCLRGDTDADRPRVEPRAQRAVVTESSLYGEHLAQPGTLKEVWSQL QSISNYLQTQDQTDQQEAEWLVLLSRFDRLLFQSYLFMLGIYTITLCSLWALWGGV (SEQ ID NO:57).

**[0115]** In some embodiments, the wild-type LGIC receptor is a human Gamma-aminobutyric acid receptor A (GABA-A), subunit beta-3 (GABA-A β3) (GenBank Accession No. NP_000805.1, SEQ ID NO:8), encoded by the *GABRB3* gene (GenBank Accession No. NM_000814.5, SEQ ID NO:7):

**(a)** *MWGLAGGRLFGIFSAPVLVAVVCCA***QSVNDPGNMSFVKETVDKLLKG YDIRLRPDFGGPPVCVGMNIDIASIDMVSEVNMDYTLTMYFQQYWRDKRLAYS GIPLNLTLDNRVADQLWVPDTYFLNDKKSFVHGVTVKNRMIRLHPDGTVLYGL RITTTAACMMDLRRYPLDEQNCTLEIESYGYTTDDIEFYWRGGDKAVTGVERIE LPQFSIVEHRLVSRNVVFATGAYPRLSLSFRLKRNIGY**FILQTYMPSILITILSWVSF WINYDASAARVALGITTVLTMTTINTHLRETLPKIPYVKAIDMYLMGCFVFVFLALL EYAFVNYIFFGRGPQRQKKLAEKTAKAKNDRSKSESNRVDAHGNILLTSLEVHNEM NEVSGGIGDTRNSAISFDNSGIQYRKQSMPREGHGRFLGDRSLPHKKTHLRRRSSQLK IKIPDLTDVNAIDRWSRIVFPFTFSLFNLVYWLYYVN (SEQ ID NO:8).

**[0116]** In some embodiments, the wild-type LGIC receptor is a human GABA-A, subunit rho1 (ρ1) (GABA-A ρ1) (GenBank Accession No. NP_002033.2, SEQ ID NO:10), encoded by the *GABRR1* gene (GenBank Accession No. NM_002042.4, SEQ ID NO:9):

**(a)** *MLAVPNMRFGIFLLWWGWVLA***TESRMHWPGREVHEMSKKGRPQRQ RREVHEDAHKQVSPILRRSPDITKSPLTKSEQLLRIDDHDFSMRPGFGGPAIPVG VDVQVESLDSISEVDMDFTMTLYLRHYWKDERLSFPSTNNLSMTFDGRLVKKI WVPDMFFVHSKRSFIHDTTTDNVMLRVQPDGKVLYSLRVTVTAMCNMDFSRFP LDTQTCSLEIESYAYTEDDLMLYWKKGNDSLKTDERISLSQFLIQEFHTTTKLAF YSSTGWYNRLYINFTLRRHIFF**FLLQTYFPATLMVMLSWVSFWIDRRAVPARVPLG ITTVLTMSTIITGVNASMPRVSYIKAVDIYLWVSFVFVFLSVLEYAAVNYLTTVQERK EQKLREKLPCTSGLPPPRTAMLDGNYSDGEVNDLDNYMPENGEKPDRMMVQLTLA SERSSPQRKSQRSSYVSMRIDTHAIDKYSRIIFPAAYILFNLIYWSIFS (SEQ ID NO:10).

**[0117]** In some embodiments, the wild-type LGIC receptor is a human GABA-A, subunit rho2 (ρ2) (GABA-A ρ2) (GenBank Accession No. NP_002034.3, SEQ ID NO:12), encoded by the GABRR2 gene (GenBank Accession No. NM_002043.4, SEQ ID NO:11):

**(a)** *MPYFTRLILFLFCLMVLVES***RKPKRKRWTGQVEMPKPSHLYKKNLD VTKIRKGKPQQLLRVDEHDFSMRPAFGGPAIPVGVDVQVESLDSISEVDMDFTM TLYLRHYWKDERLAFSSASNKSMTFDGRLVKKIWVPDVFFVHSKRSFTHDTTT DNIMLRVFPDGHVLYSMRITVTAMCNMDFSHFPLDSQTCSLELESYAYTDEDLM LYWKNGDESLKTDEKISLSQFLIQKFHTTSRLAFYSSTGWYNRLYINFTLRRHIF F**FLLQTYFPATLMVMLSWVSFWIDRRAVPARVSLGITTVLTMTTIITGVNASMPRVS YVKAVDIYLWVSFVFVFLSVLEYAAVNYLTTVQERKERKLREKFPCMCGMLHSKT MMLDGSYSESEANSLAGYPRSHILTEEERQDKIVVHLGLSGEANAARKKGLLKGQT GFRIFQNTHAIDKYSRLIFPASYIFFNLIYWSVFS (SEQ ID NO:12).

**[0118]** In some embodiments, the wild-type LGIC receptor is a human GABA-A, subunit rho3 (ρ3) (GABA-A ρ3) (GenBank Accession No. NP_001099050.1, SEQ ID NO: 14), encoded by the GABRR3 gene (GenBank Accession No. NM_001105580.2, SEQ ID NO:13):

**(a)** *MVLAFQLVSFTYIWIILKPNVCA***ASNIKMTHQRCSSSMKQTCKQETRM KKDDSTKARPQKYEQLLHIEDNDFAMRPGFGGSPVPVGIDVHVESIDSISETNMD FTMTFYLRHYWKDERLSFPSTANKSMTFDHRLTRKIWVPDIFFVHSKRSFIHDT TMENIMLRVHPDGNVLLSLRITVSAMCFMDFSRFPLDTQNCSLELESYAYNEDD LMLYWKHGNKSLNTEEHMSLSQFFIEDFSASSGLAFYSSTGWYNRLFINFVLRR HVFF**FVLQTYFPAILMVMLSWVSFWIDRRAVPARVSLGITTVLTMSTIITAVSASMPQ

VSYLKAVDVYLWVSSLFVFLSVIEYAAVNYLTTVEERKQFKKTGKISRMYNIDAVQ AMAFDGCYHDSEIDMDQTSLSLNSEDFMRRKSICSPSTDSSRIKRRKSLGGHVGRIILE NNHVIDTYSRILFPIVYILFNLFYWGVYV (SEQ ID NO:14).

**[0119]** In some aspects, the engineered receptor is a chimeric LGIC receptor. In some embodiments, the chimeric receptor comprises a ligand binding domain sequence derived from at least a first LGIC and an ion pore conduction domain sequence, or more simply, "ion pore domain sequence" derived from at least a second LGIC. In some embodiments, the derived amino acid sequence is identical to the corresponding region of the LGIC from which it was derived. In some embodiments, the derived amino acid sequence may contain alterations in at least one amino acid position compared to the corresponding region of the LGIC from which it was derived. In some embodiments, an amino acid sequence derived from an LGIC sequence differs by up to 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid residues from the corresponding region of the original amino acid sequence. In some embodiments, a derived amino acid sequence has at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% (including all ranges and subranges therebetween) sequence identity to the corresponding region of the LGIC amino acid sequence.

**[0120]** In some embodiments, the first and second LGIC are Cys-loop receptors. Ligand binding domain sequences and ion pore domain sequences of the Cys-loop receptors are well known in the art and can be readily identified from the literature by use of publicly available software, *e.g.* PubMed, Genbank, Uniprot, and the like. In some embodiments, the ligand binding domain of the chimeric receptor has at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% sequence identity to the ligand binding domain of the first LGIC. In some embodiments, the ion pore domain of the chimeric receptor has at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% sequence identity to the ion pore domain of the second LGIC. In the sequences described above, the ligand binding domain is bolded, and the ion pore domain is underlined.

**[0121]** In some embodiments, the ligand binding domain of the chimeric receptor is derived from the ligand binding domain sequence of a human glycine receptor. In some embodiments, the human glycine receptor is human GlyRα1 (SEQ ID NO:2). In some embodiments, the ligand binding domain comprises an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% sequence identity to about amino acids 29-235 of GlyRα1, *e.g.* amino acids

29-235, amino acids 29-240, amino acids 29-246, amino acids 29-248, amino acids 29-250, or amino acids 29-252 of SEQ ID NO:2. In certain such embodiments, the ligand binding domain consists essentially of amino acids 29-235 of SEQ ID NO:2, consists essentially of amino acids 29-240 of SEQ ID NO:2, consists essentially of amino acids 29-246 of SEQ ID NO:2, consists essentially of amino acids 29-248 of SEQ ID NO:2, consists essentially of amino acids 29-250 of SEQ ID NO:2, consists essentially of amino acids 29-252 of SEQ ID NO:2. In some embodiments, the ion pore domain sequence is derived from a Cys-loop receptor other than the human GlyRα1.

**[0122]** In some embodiments, the ligand binding domain of the chimeric receptor comprises the ligand binding domain sequence of a human nicotinic cholinergic receptor. In some embodiments, the human nicotinic cholinergic receptor is human α7-nAChR. In some embodiments, the ligand binding domain comprises about amino acids 23-220 of human α7-nAChR (SEQ ID NO:4), *e.g.* amino acids 23-220, amino acids 23-221, amino acids 23-222, amino acids 23-223, amino acids 23-224, amino acids 23-225, amino acids 23-226, amino acids 23-227, amino acids 23-228, amino acids 23-229, amino acids 23-230, or amino acids 23-231 of SEQ ID NO:4. In some embodiments, the ligand binding domain consists essentially of amino acids 23-220, amino acids 23-221, amino acids 23-222, amino acids 23-223, amino acids 23-224, amino acids 23-225, amino acids 23-226, amino acids 23-227, amino acids 23-228, amino acids 23-229, amino acids 23-230, or amino acids 23-231 of SEQ ID NO: 4. In some embodiments, the ion pore domain sequence is derived from a Cys-loop receptor other than the human α7-nAChR.

**[0123]** In some embodiments, the ligand binding domain of the chimeric receptor is derived from the ligand binding domain sequence of a human nicotinic cholinergic receptor. In some embodiments, the human nicotinic cholinergic receptor is human α7-nAChR. In some embodiments, the ligand binding domain comprises an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% sequence identity to about amino acids 23-220 of human α7-nAChR (SEQ ID NO: 4), e.g. amino acids 23-220, amino acids 23-221, amino acids 23-222, amino acids 23-223, amino acids 23-224, amino acids 23-225, amino acids 23-226, amino acids 23-227, amino acids 23-228, amino acids 23-229, amino acids 23-230, or amino acids 23-231 of SEQ ID NO: 4. In some embodiments, the ion pore domain sequence is derived from a Cys-loop receptor other than the human α7-nAChR.

**[0124]** In some embodiments, the ligand binding domain of the chimeric receptor is derived from the ligand binding domain sequence of a human serotonin receptor. In some

embodiments, the human serotonin receptor is human 5HT3A or 5HT3B. In some such embodiments, the ligand binding domain comprises about amino acids 23-247 of 5HT3A (SEQ ID NO:6), *e.g.* amino acids 23-240, amino acids 30-245, amino acids 23-247, amino acids 23-250, in some instances amino acids 30-255 of SEQ ID NO:6. In certain embodiments, the ligand binding domain consists essentially of amino acids 23-240 of SEQ ID NO:6, consists essentially of amino acids 23-245 of SEQ ID NO:6, consists essentially of amino acids 30-247 of SEQ ID NO:6, consists essentially of amino acids 23-250 of SEQ ID NO:6, consists essentially of amino acids 23-255 of SEQ ID NO:6. In some such embodiments, the ligand binding domain comprises about amino acids 21-239 of 5HT3B (SEQ ID NO:57), *e.g.* amino acids 21-232, amino acids 21-235, amino acids 21-240, amino acids 21-245, in some instances amino acids 21-247 of SEQ ID NO:57. In certain embodiments, the ligand binding domain consists essentially of amino acids 21-239 of SEQ ID NO:57, consists essentially of amino acids 21-232 of SEQ ID NO:57, consists essentially of amino acids 21-235 of SEQ ID NO:57, consists essentially of amino acids 21-240 of SEQ ID NO:57, consists essentially of amino acids 21-245 of SEQ ID NO:57. In some embodiments, the ion pore domain sequence is derived from a Cys-loop receptor other than the human 5-hydroxytryptamine receptor 3.

**[0125]** In some embodiments, the ligand binding domain of the chimeric receptor is derived from the ligand binding domain sequence of a human GABA receptor. In some embodiments, the human GABA receptor is human GABA-A β3. In some such embodiments, the ligand binding domain comprises about amino acids 26-245 of GABA-A β3 (SEQ ID NO:8), *e.g.* amino acids 26-240, amino acids 26-245, amino acids 26-248, amino acids 26-250, in some instances amino acids 26-255 of SEQ ID NO:8. In certain such embodiments, the ligand binding domain consists essentially of amino acids 26-240 of SEQ ID NO:8, consists essentially of amino acids 26-245 of SEQ ID NO:8, consists essentially of amino acids 26-248 of SEQ ID NO:8, consists essentially of amino acids 26-250 of SEQ ID NO:8, or consists essentially of amino acids 26-255 of SEQ ID NO:8. In some embodiments, the ion pore domain sequence is derived from a Cys-loop receptor other than the human GABA-A receptor.

**[0126]** In some embodiments, the ion pore domain to which the ligand binding domain is fused conducts anions, *e.g.* it comprises an ion pore domain sequence of a human glycine receptor or a human serotonin receptor. In other embodiments, the ion conduction pore domain to which the ligand binding domain is fused conducts cations, *e.g.* it comprises an ion pore domain sequence of a human acetylcholine receptor or a human gamma-aminobutyric acid receptor A.

**[0127]** In some embodiments, the ion pore domain of the engineered receptor is derived from the ion pore domain sequence of a human glycine receptor. In some embodiments, the human glycine receptor is human GlyRα1. In some embodiments, the ion pore domain comprises an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% sequence identity to about amino acids 245-457 of GlyRα1 (SEQ ID NO:2), *e.g.* amino acids 240-457, amino acids 245-457, amino acids 248-457, amino acids 249-457, amino acids 250-457, amino acids 255-457, or amino acids 260-457 of SEQ ID NO:2. In some embodiments, the ion pore domain consists essentially of amino acids 245-457 of SEQ ID NO:2, consists essentially of amino acids 248-457 of SEQ ID NO:2, consists essentially of amino acids 249-457 of SEQ ID NO:2, or consists essentially of amino acids 250-457 of SEQ ID NO:2.

**[0128]** In some embodiments, the ion pore domain of the chimeric receptor comprises the ion pore domain sequence of human GlyRα2 (SEQ ID NO: 83). In some embodiments, the ion pore domain of the chimeric receptor comprises, consists essentially of, or consists of an amino acid sequence derived from the ion pore domain sequence of human GlyRα2 (SEQ ID NO: 83). In some embodiments, the ion pore domain of the chimeric receptor comprises, consists essentially of, or consists of an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% sequence identity to the ion pore domain sequence of human GlyRα2 (SEQ ID NO: 83). In some embodiments, the ion pore domain of the chimeric receptor comprises, consists essentially of, or consists of an amino acid sequence identical to the ion pore domain sequence of human GlyRα2 (SEQ ID NO: 83). In some embodiments, the ion pore domain sequence of human GlyRα2 comprises, consists essentially of, or consists of amino acids 254-452 of SEQ ID NO: 83. In some embodiments, the ion pore domain sequence of human GlyRα2 comprises, consists essentially of, or consists of amino acids 254-452 of SEQ ID NO: 83. In some embodiments, the ion pore domain sequence of human GlyRα2 comprises, consists essentially of, or consists of amino acids 258-452 of SEQ ID NO: 83. In some embodiments, the ion pore domain sequence of human GlyRα2 comprises, consists essentially of, or consists of amino acids 260-452 of SEQ ID NO: 83.

**[0129]** In some embodiments, the ion pore domain of the chimeric receptor comprises the ion pore domain sequence of human GlyRα3 isoform L (SEQ ID NO: 85). In some embodiments, the ion pore domain of the chimeric receptor comprises, consists essentially of, or consists of an amino acid sequence derived from the ion pore domain sequence of human GlyRα3 isoform L (SEQ ID NO: 85). In some embodiments, the ion pore domain of the

chimeric receptor comprises, consists essentially of, or consists of an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% sequence identity to the ion pore domain sequence of human GlyRα3 isoform L (SEQ ID NO: 85). In some embodiments, the ion pore domain of the chimeric receptor comprises, consists essentially of, or consists of an amino acid sequence identical to the ion pore domain sequence of human GlyRα3 isoform L (SEQ ID NO: 85). In some embodiments, the ion pore domain sequence of human GlyRα3 isoform L comprises, consists essentially of, or consists of amino acids 253-464 of SEQ ID NO: 85. In some embodiments, the ion pore domain sequence of human GlyRα3 isoform L comprises, consists essentially of, or consists of amino acids 257-464 of SEQ ID NO: 85. In some embodiments, the ion pore domain sequence of human GlyRα3 isoform L comprises, consists essentially of, or consists of amino acids 259-464 of SEQ ID NO: 85.

**[0130]** In some embodiments, the ion pore domain of the chimeric receptor comprises the ion pore domain sequence of human GlyRα3 isoform K (SEQ ID NO: 87). In some embodiments, the ion pore domain of the chimeric receptor comprises, consists essentially of, or consists of an amino acid sequence derived from the ion pore domain sequence of human GlyRα3 isoform K (SEQ ID NO: 87). In some embodiments, the ion pore domain of the chimeric receptor comprises, consists essentially of, or consists of an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% sequence identity to the ion pore domain sequence of human GlyRα3 isoform K (SEQ ID NO: 87). In some embodiments, the ion pore domain of the chimeric receptor comprises, consists essentially of, or consists of an amino acid sequence identical to the ion pore domain sequence of human GlyRα3 isoform K (SEQ ID NO: 87). In some embodiments, the ion pore domain sequence of human GlyRα3 isoform K comprises, consists essentially of, or consists of amino acids 253-449 of SEQ ID NO: 87. In some embodiments, the ion pore domain sequence of human GlyRα3 isoform K comprises, consists essentially of, or consists of amino acids 257-449 of SEQ ID NO: 87. In some embodiments, the ion pore domain sequence of human GlyRα3 isoform K comprises, consists essentially of, or consists of amino acids 259-449 of SEQ ID NO: 87.

**[0131]** In some embodiments, the ion pore domain is derived from the ion pore domain sequence of a human nicotinic cholinergic receptor. In some embodiments, the human nicotinic cholinergic receptor is human α7-nAChR. In some embodiments, the ion pore domain comprises an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% sequence identity to about

amino acids 230-502 of α7-nAChR (SEQ ID NO:4), *e.g.* amino acids 227-502, amino acids 230-502, amino acids 231-502, amino acids 232-502, or amino acids 235-502. In certain such embodiments, the ion pore domain consists essentially of amino acids 227-502 of SEQ ID NO:4, consists essentially of amino acids 230-502 of SEQ ID NO:4, consists essentially of amino acids 231-502 of SEQ ID NO:4, consists essentially of amino acids 232-502 of SEQ ID NO:4, or consists essentially of amino acids 235-502 of SEQ ID NO:4.

**[0132]** In some embodiments, the ion pore domain is derived from the ion pore domain sequence of a human serotonin receptor. In some embodiments, the human serotonin receptor is human 5HT3A or 5HT3B. In some such embodiments, the ion pore domain comprises an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% sequence identity to about amino acids 248-516 of 5HT3A (SEQ ID NO:6), *e.g.* amino acids 240-516, amino acids 245-516, amino acids 248-516, amino acids 250-516, or amino acids 255-516 of SEQ ID NO:6. In certain such embodiments, the ion pore domain consists essentially of amino acids 240-516 of SEQ ID NO:6, consists essentially of amino acids 245-516 of SEQ ID NO:6, consists essentially of amino acids 248-516 of SEQ ID NO:6, consists essentially of amino acids 250-516 of SEQ ID NO:6, or consists essentially of amino acids 253-516. In some embodiments, the ion pore domain comprises an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% sequence identity to about amino acids 240-441 of 5HT3B (SEQ ID NO:57), *e.g.* amino acids 230-441, amino acids 235-441, amino acids 240-441, amino acids 245-441, or amino acids 250-441 of SEQ ID NO:57. In certain such embodiments, the ion pore domain consists essentially of amino acids 230-441 of SEQ ID NO:57, consists essentially of amino acids 235-441 of SEQ ID NO:57, consists essentially of amino acids 240-441 of SEQ ID NO:57, consists essentially of amino acids 245-441 of SEQ ID NO:57, or consists essentially of amino acids 250-441.

**[0133]** In some embodiments, the ion pore domain is derived from the ion pore domain sequence of a human GABA receptor. In some embodiments, the human GABA receptor is human GABA-A β3. In some embodiments, the ion pore domain comprises an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% sequence identity to about amino acids 246-473 of GABA-A β3 (SEQ ID NO:8), *e.g.* amino acids 240-473, amino acids 245-473, amino acids 247-473, amino acids 250-473, or amino acids 253-473 of SEQ ID NO:8. In certain such embodiments, the ion pore domain consists essentially of amino acids 240-473 of SEQ ID

NO:8, amino acids 245-473 of SEQ ID NO:8, amino acids 247-473 of SEQ ID NO:8, amino acids 250-473 of SEQ ID NO:8, or amino acids 253-473 of SEQ ID NO:8.

**[0134]** In some embodiments, the ion pore domain of the chimeric receptor comprises the ion pore domain sequence of human GABA-A ρ1 (GABRR1, SEQ ID NO: 10). In some embodiments, the ion pore domain of the chimeric receptor comprises, consists essentially of, or consists of an amino acid sequence derived from the ion pore domain sequence of human GABA-A ρ1 (SEQ ID NO: 10). In some embodiments, the ion pore domain of the chimeric receptor comprises, consists essentially of, or consists of an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% sequence identity to the ion pore domain sequence of human GABA-A ρ1 (SEQ ID NO: 10). In some embodiments, the ion pore domain of the chimeric receptor comprises, consists essentially of, or consists of an amino acid sequence identical to the ion pore domain sequence of human GABA-A ρ1 (SEQ ID NO: 10). In some embodiments, the ion pore domain sequence of human GABA-A ρ1 comprises, consists essentially of, or consists of amino acids 284-479 of SEQ ID NO: 10. In some embodiments, the ion pore domain sequence of human GABA-A ρ1 comprises, consists essentially of, or consists of amino acids 288-479 of SEQ ID NO: 10. In some embodiments, the ion pore domain sequence of human GABA-A ρ1 comprises, consists essentially of, or consists of amino acids 290-479 of SEQ ID NO: 10.

**[0135]** In some embodiments, the ion pore domain of the chimeric receptor comprises the ion pore domain sequence of human GABA-A ρ2 (GABRR2, SEQ ID NO: 12). In some embodiments, the ion pore domain of the chimeric receptor comprises, consists essentially of, or consists of an amino acid sequence derived from the ion pore domain sequence of human GABA-A ρ2 (SEQ ID NO: 12). In some embodiments, the ion pore domain of the chimeric receptor comprises, consists essentially of, or consists of an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% sequence identity to the ion pore domain sequence of human GABA-A ρ2 (SEQ ID NO: 12). In some embodiments, the ion pore domain of the chimeric receptor comprises, consists essentially of, or consists of an amino acid sequence identical to the ion pore domain sequence of human GABA-A ρ2 (SEQ ID NO: 12). In some embodiments, the ion pore domain sequence of human GABA-A ρ2 comprises, consists essentially of, or consists of amino acids 265-466 of SEQ ID NO: 12. In some embodiments, the ion pore domain sequence of human GABA-A ρ2 comprises, consists essentially of, or consists of amino acids 269-466 of SEQ ID NO: 12. In some embodiments, the ion pore domain sequence of human GABA-A ρ2 comprises, consists essentially of, or consists of amino acids 271-466 of SEQ ID NO: 12.

**[0136]** In some embodiments, the ion pore domain of the chimeric receptor comprises the ion pore domain sequence of human GABA-A ρ3 (GABRR3, SEQ ID NO: 14). In some embodiments, the ion pore domain of the chimeric receptor comprises, consists essentially of, or consists of an amino acid sequence derived from the ion pore domain sequence of human GABA-A ρ3 (SEQ ID NO: 14). In some embodiments, the ion pore domain of the chimeric receptor comprises, consists essentially of, or consists of an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% sequence identity to the ion pore domain sequence of human GABA-A ρ3 (SEQ ID NO: 14). In some embodiments, the ion pore domain of the chimeric receptor comprises, consists essentially of, or consists of an amino acid sequence identical to the ion pore domain sequence of human GABA-A ρ3 (SEQ ID NO: 14). In some embodiments, the ion pore domain sequence of human GABA-A ρ3 comprises, consists essentially of, or consists of amino acids 271-468 of SEQ ID NO: 14. In some embodiments, the ion pore domain sequence of human GABA-A ρ3 comprises, consists essentially of, or consists of amino acids 275-468 of SEQ ID NO: 14. In some embodiments, the ion pore domain sequence of human GABA-A ρ3 comprises, consists essentially of, or consists of amino acids 277-467 of SEQ ID NO: 14.

**[0137]** In some embodiments, the ion pore domain of the subject chimeric ligand-gated ion channel comprises an M2-M3 linker domain that is heterologous to the M2-M3 linker domain of the ion pore domain. By an "M2-M3 linker domain", or "M2-M3 linker", it is meant the sequence within an ion pore domain of a LGIC that is flanked at its amino (N) terminus by the C-terminal end of transmembrane domain 2 (M2) of the receptor and at its carboxy (C) terminus by the N-terminal end of transmembrane domain 3 (M3) of the receptor. The M2-M3 linker of a LGIC may be readily determined from the art and/or by using any publicly available protein analysis tool, *e.g.* Expasy, uniProt, etc. In some embodiments,, when the ion pore domain of a chimeric receptor comprises a heterologous M2-M3 linker, the M2-M3 linker is derived from the same receptor as the ligand binding domain of the chimeric receptor. For example, when the subject ligand-gated ion channel comprises a ligand binding domain from an AChR and an ion pore domain from a GlyR, its ion pore domain sequence may comprise a M2-M3 linker sequence derived from the AChR. In some embodiments, the ion pore domain is derived from GlyRα1 and the M2-M3 linker is derived from α7-nAChR. In some embodiments, the native M2-M3 linker sequence that is removed from the ion pore domain corresponds to about amino acids 293-313, of GlyRα1 (SEQ ID NO:2), *e.g.* amino acids 304-310, 293-306, 298-310, 305-311, 302-313, etc. In some such embodiments, the M2-M3 linker that is inserted is derived from about amino acids 281-295 of α7-nAChR (SEQ ID NO:4), *e.g.*

amino acids 290-295, 281-290, 281-295, 283-295, 287-292, etc. or a sequence at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% , or 100% identical to amino acids 281-295 or 283-295 of α7-nAChR.

**[0138]** In some embodiments, the ligand binding domain of the subject chimeric ligand-gated ion channel comprises a Cys-loop domain sequence that is heterologous to the Cys-loop sequence of the ligand binding domain. By a "Cys-loop domain sequence", or "Cys-loop sequence", it is meant the domain within a ligand binding domain of a Cys-loop LGIC that forms a loop structure flanked by a cysteine at the N-terminus and the C-terminus. Without wishing to be bound by theory, it is believed that upon binding of the ligand to the ligand binding domain, the Cys-loop structurally moves to be in close proximity to the M2-M3 loop, this movement mediating the biophysical translation of ligand binding in the extracellular domain to signal transduction in the ion pore domain (as reviewed in Miller and Smart, Trends in Pharmacological Sci 2009:31(4)). The substitution of an endogenous Cys-loop sequence with a heterologous Cys-loop sequence may increase the conductivity of the LGIC by 1.5-fold or more, *e.g.* at least 2-fold, 3-fold or 4-fold, in some instances at least 5-fold or 6-fold, and at certain doses, at least 7-fold, 8-fold, 9-fold or 10-fold. The Cys-loop domain of a Cys-loop receptor may be readily determined from the art and/or by using any publicly available protein analysis tool, *e.g.* Expasy, uniProt, etc. Typically, when the ligand binding domain of a chimeric receptor comprises a heterologous Cys-loop sequence, the Cys-loop sequence is derived from the same receptor as the ion pore domain of the chimeric receptor. For example, when the subject chimeric ligand-gated ion channel comprises a ligand binding domain from an AChR and an ion pore domain from a GlyR, the subject ligand-gated ion channel may comprise ligand binding domain sequence from an AChR except for the sequence of the Cys-loop domain, which is instead derived from a GlyR. In some embodiments, the ligand binding domain is derived from α7-nAChR and the Cys-loop sequence is derived from a GLyR. In some embodiments, the Cys-loop sequence that is removed from the ligand binding domain corresponds to about amino acids 150-164 of α7-nAChR (SEQ ID NO:4), *e.g.* amino acids 150-157 of α7-nAChR. In some embodiments, the Cys loop sequence that is inserted is derived from about amino acids 166-180 of GlyRα1 (SEQ ID NO:2), *e.g.* amino acids 166-172 of GlyRα1, or a sequence at least 80%, at least 85%, at least 90%, or at least 95% identical to amino acids 166-180 of GlyRα1.

**[0139]** In some embodiments, the Cys loop sequence that is inserted is derived from about amino acids 172-186 of GlyRα2 (SEQ ID NO:83), e.g. amino acids 172-178 of GlyRα2, or a sequence at least 80%, at least 85%, at least 90%, or at least 95% identical to amino acids

172-186 of GlyRα2. In some embodiments, the Cys loop sequence that is inserted is derived from about amino acids 171-185 of GlyRα3 (SEQ ID NO:85 or 87), e.g. amino acids 171-177 of GlyRα3, or a sequence at least 80%, at least 85%, at least 90%, or at least 95% identical to amino acids 171-185 of GlyRα3. In some embodiments, the Cys loop sequence that is inserted is derived from about amino acids 198-212 of GABA-A ρ1 (SEQ ID NO:10), e.g. amino acids 198-204 of GABA-A ρ1, or a sequence at least 80%, at least 85%, at least 90%, or at least 95% identical to amino acids 198-212 of GABA-A ρ1. In some embodiments, the Cys loop sequence that is inserted is derived from about amino acids 178-192 of GABA-A ρ2 (SEQ ID NO:12), e.g. amino acids 178-184 of GABA-A ρ2, or a sequence at least 80%, at least 85%, at least 90%, or at least 95% identical to amino acids 178-192 of GABA-A ρ2. In some embodiments, the Cys loop sequence that is inserted is derived from about amino acids 184-198 of GABA-A ρ3 (SEQ ID NO:14), e.g. amino acids 184-190 of GABA-A ρ3, or a sequence at least 80%, at least 85%, at least 90%, or at least 95% identical to amino acids 184-198 of GABA-A ρ3.

**[0140]** In some embodiments, the ligand binding domain of the subject chimeric ligand-gated ion channel comprises a β1-2 loop domain sequence that is heterologous to the β1-2 loop domain sequence of the ligand binding domain. By a "β1-2 loop domain sequence", or "β1-2 loop, or β1- β2 loop", it is meant the domain within a ligand binding domain of a Cys-loop LGIC that is flanked at its N-terminus by the C-terminus of the β1 sheet and, at its C-terminus, by the N-terminus of the β2 sheet. Without wishing to be bound by theory, it is believed that the β1-2 loop helps to mediate biophysical translation of ligand binding in the extracellular domain to the ion pore domain and subsequent signal transduction (i.e. chloride influx in case of GlyR). It is believed that upon binding of ligand, the β1-2 loop, together with the Cys-loop, come in close proximity to the M2-M3 loop to mediate the biophysical translation of ligand binding in the extracellular domain to signal transduction in the ion pore domain where the M2-M3 loop resides (as reviewed in Miller and Smart, *supra*). The substitution of an endogenous β1-2 loop sequence with a heterologous β1-2 loop sequence may increase the conductivity of the LGIC by 1.5-fold or more, *e.g.* at least 2-fold, 3-fold or 4-fold, in some instances at least 5-fold or 6-fold, and at certain doses, at least 7-fold, 8-fold, 9-fold or 10-fold. The β1-2 loop of a Cys-loop receptor may be readily determined from the art and/or by using any publicly available protein analysis tool, *e.g.* Expasy, uniProt, etc. Typically, when the ligand binding domain of a chimeric receptor comprises a heterologous β1-2 loop sequence, the β1-2 loop sequence is derived from the same receptor as the ion pore domain of the chimeric receptor. For example, when the subject chimeric ligand-gated ion channel comprises a ligand binding domain derived from an AChR and an ion pore domain derived from a GlyR, the

sequence of the β1-2 loop domain of the ligand binding domain may be derived from a GlyR. In some embodiments, the ligand binding domain is derived from α7-nAChR. In some embodiments, the β1-2 loop sequence that is removed from the ligand binding domain corresponds to about amino acids 64-72 or 67-70 of α7-nAChR (SEQ ID NO:4), *e.g.* amino acids 67-70, 66-71 or 64-72 of α7-nAChR. In some embodiments, the β1-2 loop sequence that is inserted is about amino acids 79-85 of GlyRα1 (SEQ ID NO:2), *e.g.* amino acids 80-85, 81-84, 79-85, or 81-84 of GlyRα1, with at most 3, at most 2, at most 1, or no amino acid mutations. In some embodiments, the ion pore domain is derived from GlyRα2 and the β1-2 loop that is inserted corresponds to about amino acids 86-91 of GlyRα2 (SEQ ID NO:83) with at most 3, at most 2, at most 1, or no amino acid mutations. In some embodiments, the ion pore domain is derived from GlyRα3 and the β1-2 loop that is inserted corresponds to about amino acids 85-90 of GlyRα3 (SEQ ID NO:85 or 87) with at most 3, at most 2, at most 1, or no amino acid mutations. In some embodiments, the ion pore domain is derived from GABA-A ρ1 and the β1-2 loop that is inserted corresponds to about amino acids 112-117 of GABA-A ρ1 (SEQ ID NO:10) with at most 3, at most 2, at most 1, or no amino acid mutations. In some embodiments, the ion pore domain is derived from GABA-A ρ2 and the β1-2 loop that is inserted corresponds to about amino acids 92-97 of GABA-A ρ2 (SEQ ID NO:12) with at most 3, at most 2, at most 1, or no amino acid mutations. In some embodiments, the ion pore domain is derived from GABA-A ρ3 and the β1-2 loop that is inserted corresponds to about amino acids 98-103 of GABA-A ρ3 (SEQ ID NO:14) with at most 3, at most 2, at most 1, or no amino acid mutations. In some embodiments, the mutation is an amino acid substitution.

**[0141]** In some embodiments, the disclosure provides chimeric LGIC receptors comprising a ligand binding domain derived from human α7-nAChR, wherein the ligand binding domain comprises one or more amino acid substitutions of the disclosure, and an ion pore domain derived from a human Glycine receptor. In some embodiments, the human Glycine receptor is human Glycine receptor α1, human Glycine receptor α2, or human Glycine receptor α3. In some embodiments, the ligand binding domain comprises a Cys-loop domain derived from the human Glycine receptor. In some embodiments, the ligand binding domain comprises a β1-2 loop domain derived from the human Glycine receptor.

**[0142]** In some embodiments, the disclosure provides chimeric LGIC receptors comprising a ligand binding domain derived from human α7-nAChR, wherein the ligand binding domain comprises one or more amino acid substitutions of the disclosure, and an ion pore domain derived from a human GABA receptor. In some embodiments, the human GABA receptor is human GABA-A ρ1, human GABA-A ρ2, or human GABA-A ρ3. In some

embodiments, the ligand binding domain comprises a Cys-loop domain derived from the human GABA receptor. In some embodiments, the ligand binding domain comprises a β1-2 loop domain derived from the human GABA receptor.

**[0143]** Non-limiting examples of sequences of chimeric LGIC receptors of the present disclosure include the sequences disclosed herein as SEQ ID NO:15 – SEQ ID NO:52. In some embodiments, the chimeric LGIC receptor or the polynucleotide that encodes it has a sequence identity of 85% or more to a sequence provided in SEQ ID NO:15 – SEQ ID NO:52 herein, e.g. a sequence identity of 90% or more, 93% or more, or 95% or more, i.e. about 96%, about 97%, about 98%, about 99% or about 100% to a sequence provided in SEQ ID NO:15 - SEQ ID NO:52. In the sequences, the signal peptide is italicized, the ligand binding domain is bolded, and the ion pore domain is underlined.

**[0144]** In some embodiments, the chimeric LGIC receptor is a CHRNA7/GLRA1 chimera (R229 junction), comprising the human α7-nAChR signal peptide (italics) and ligand binding domain (bold), fused to the human GlyRα1 ion pore domain (underlined):

*MRCSPGGVWLALAASLLHVSLQ***GEFQRKLYKELVKNYNPLERPVANDSQPL TVYFSLSLLQIMDVDEKNQVLTTNIWLQMSWTDHYLQWNVSEYPGVKT VRFPDGQIWKPDILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSSC YIDVRWFPFDVQHCKLKFGSWSYGGWSLDLQMQEADISGYIPNGEWDL VGIPGKRSERFYECCKEPYPDVTFTVTMRRR**MGYYLIQMYIPSLLIVILSWI SFWINMDAAPARVGLGITTVLTMTTQSSGSRASLPKVSYVKAIDIWMAVCLL FVFSALLEYAAVNFVSRQHKELLRFRRKRRHHKSPMLNLFQEDEAGEGRFNF SAYGMGPACLQAKDGISVKGANNSNTTNPPPAPSKSPEEMRKLFIQRAKKID KISRIGFPMAFLIFNMFYWIIYKIVRREDVHNQ (SEQ ID NO:16, encoded by SEQ ID NO:15).

**[0145]** In some embodiments, the chimeric LGIC receptor is a CHRNA7/GLRA1 (R228 junction) chimera, comprising the human α7-nAChR signal peptide (italics) and ligand binding domain (bold), fused to the human GlyRα1 ion pore domain (underlined):

*MRCSPGGVWLALAASLLHVSLQ***GEFQRKLYKELVKNYNPLERPVANDSQPL TVYFSLSLLQIMDVDEKNQVLTTNIWLQMSWTDHYLQWNVSEYPGVKT VRFPDGQIWKPDILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSSC YIDVRWFPFDVQHCKLKFGSWSYGGWSLDLQMQEADISGYIPNGEWDL VGIPGKRSERFYECCKEPYPDVTFTVTMRRQ**MGYYLIQMYIPSLLIVILSWI SFWINMDAAPARVGLGITTVLTMTTQSSGSRASLPKVSYVKAIDIWMAVCLL

FVFSALLEYAAVNFVSRQHKELLRFRRKRRHHKSPMLNLFQEDEAGEGRFNF SAYGMGPACLQAKDGISVKGANNSNTTNPPPAPSKSPEEMRKLFIQRAKKID KISRIGFPMAFLIFNMFYWIIYKIVRREDVHNQ (SEQ ID NO:17).

**[0146]** In some embodiments, the chimeric LGIC receptor is a CHRNA7/GLRA1 (V224 junction) chimera, comprising the human α7-nAChR signal peptide (italics) and ligand binding domain (bold), fused to the human GlyRα1 ion pore domain (underlined):

*MRCSPGGVWLALAASLLHVSLQ***GEFQRKLYKELVKNYNPLERPVANDSQPL TVYFSLSLLQIMDVDEKNQVLTTNIWLQMSWTDHYLQWNVSEYPGVKT VRFPDGQIWKPDILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSSC YIDVRWFPFDVQHCKLKFGSWSYGGWSLDLQMQEADISGYIPNGEWDL VGIPGKRSERFYECCKEPYPDVTFTV**HLERQMGYYLIQMYIPSLLIVILSWIS FWINMDAAPARVGLGITTVLTMTTQSSGSRASLPKVSYVKAIDIWMAVCLLF VFSALLEYAAVNFVSRQHKELLRFRRKRRHHKSPMLNLFQEDEAGEGRFNFS AYGMGPACLQAKDGISVKGANNSNTTNPPPAPSKSPEEMRKLFIQRAKKIDKI SRIGFPMAFLIFNMFYWIIYKIVRREDVHNQ (SEQ ID NO:18).

**[0147]** In some embodiments, the chimeric LGIC receptor is a CHRNA7/GLRA1 (Y233 junction) chimera, comprising the human α7-nAChR signal peptide (italics) and ligand binding domain (bold), fused to the human GlyRα1 ion pore domain (underlined):

*MRCSPGGVWLALAASLLHVSLQ***GEFQRKLYKELVKNYNPLERPVANDSQPL TVYFSLSLLQIMDVDEKNQVLTTNIWLQMSWTDHYLQWNVSEYPGVKT VRFPDGQIWKPDILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSSC YIDVRWFPFDVQHCKLKFGSWSYGGWSLDLQMQEADISGYIPNGEWDL VGIPGKRSERFYECCKEPYPDVTFTVTMRRRTLYY**LIQMYIPSLLIVILSWIS FWINMDAAPARVGLGITTVLTMTTQSSGSRASLPKVSYVKAIDIWMAVCLLF VFSALLEYAAVNFVSRQHKELLRFRRKRRHHKSPMLNLFQEDEAGEGRFNFS AYGMGPACLQAKDGISVKGANNSNTTNPPPAPSKSPEEMRKLFIQRAKKIDKI SRIGFPMAFLIFNMFYWIIYKIVRREDVHNQ (SEQ ID NO:19).

**[0148]** In some embodiments, the chimeric LGIC receptor is a CHRNA7/GLRA1 chimera (R229 junction), comprising the human α7-nAChR signal peptide (italics) and ligand binding domain (bold), fused to the human GlyRα1 ion pore domain (underlined) comprising an α7-nAChR M2-M3 linker (lowercase):

**(a)** *MRCSPGGVWLALAASLLHVSLQ***GEFQRKLYKELVKNYNPLERPVAND SQPLTVYFSLSLLQIMDVDEKNQVLTTNIWLQMSWTDHYLQWNVSEYPGVKTV**

**RFPDGQIWKPDILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSSCYIDVR WFPFDVQHCKLKFGSWSYGGWSLDLQMQEADISGYIPNGEWDLVGIPGKRSER FYECCKEPYPDVTFTVTMRRR**MGYYLIQMYIPSLLIVILSWISFWINMDAAPARVG LGITTVLTMTTQSSGSeimpatsdsvSYVKAIDIWMAVCLLFVFSALLEYAAVNFVSRQH KELLRFRRKRRHHKSPMLNLFQEDEAGEGRFNFSAYGMGPACLQAKDGISVKGANN SNTTNPPPAPSKSPEEMRKLFIQRAKKIDKISRIGFPMAFLIFNMFYWIIYKIVRREDVH NQ (SEQ ID NO:21, encoded by SEQ ID NO:20);

**(b)** *MRCSPGGVWLALAASLLHVSLQ***GEFQRKLYKELVKNYNPLERPVAND SQPLTVYFSLSLLQIMDVDEKNQVLTTNIWLQMSWTDHYLQWNVSEYPGVKTV RFPDGQIWKPDILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSSCYIDVR WFPFDVQHCKLKFGSWSYGGWSLDLQMQEADISGYIPNGEWDLVGIPGKRSER FYECCKEPYPDVTFTVTMRRR**MGYYLIQMYIPSLLIVILSWISFWINMDAAPARVG LGITTVLTMTTQSSGSeimpatsdsvpliaqAIDIWMAVCLLFVFSALLEYAAVNFVSRQHK ELLRFRRKRRHHKSPMLNLFQEDEAGEGRFNFSAYGMGPACLQAKDGISVKGANNS NTTNPPPAPSKSPEEMRKLFIQRAKKIDKISRIGFPMAFLIFNMFYWIIYKIVRREDVHN Q (SEQ ID NO:23, encoded by SEQ ID NO:22);

**(c)** *MRCSPGGVWLALAASLLHVSLQ***GEFQRKLYKELVKNYNPLERPVAND SQPLTVYFSLSLLQIMDVDEKNQVLTTNIWLQMSWTDHYLQWNVSEYPGVKTV RFPDGQIWKPDILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSSCYIDVR WFPFDVQHCKLKFGSWSYGGWSLDLQMQEADISGYIPNGEWDLVGIPGKRSER FYECCKEPYPDVTFTVTMRRR**MGYYLIQMYIPSLLIVILSWISFWINMDAAPARVG LGITTVLTMTTQSSGSRASLPKVsdsvplIDIWMAVCLLFVFSALLEYAAVNFVSRQHKE LLRFRRKRRHHKSPMLNLFQEDEAGEGRFNFSAYGMGPACLQAKDGISVKGANNSN TTNPPPAPSKSPEEMRKLFIQRAKKIDKISRIGFPMAFLIFNMFYWIIYKIVRREDVHNQ (SEQ ID NO:25, encoded by SEQ ID NO:24);

**(d)** *MRCSPGGVWLALAASLLHVSLQ***GEFQRKLYKELVKNYNPLERPVAND SQPLTVYFSLSLLQIMDVDEKNQVLTTNIWLQMSWTDHYLQWNVSEYPGVKTV RFPDGQIWKPDILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSSCYIDVR WFPFDVQHCKLKFGSWSYGGWSLDLQMQEADISGYIPNGEWDLVGIPGKRSER FYECCKEPYPDVTFTVTM**ERQMGYYLIQMYIPSLLIVILSWISFWINMDAAPARVGL GITTVLTMTTQSSGSeimpatsdsvpliaqAIDIWMAVCLLFVFSALLEYAAVNFVSRQHKE LLRFRRKRRHHKSPMLNLFQEDEAGEGRFNFSAYGMGPACLQAKDGISVKGANNSN

TTNPPPAPSKSPEEMRKLFIQRAKKIDKISRIGFPMAFLIFNMFYWIIYKIVRREDVHNQ (SEQ ID NO:27, encoded by SEQ ID NO:26);

**(e)** *MRCSPGGVWLALAASLLHVSLQ***GEFQRKLYKELVKNYNPLERPVAND SQPLTVYFSLSLLQIMDVDEKNQVLTTNIWLQMSWTDHYLQWNVSEYPGVKTV RFPDGQIWKPDILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSSCYIDVR WFPFDVQHCKLKFGSWSYGGWSLDLQMQEADISGYIPNGEWDLVGIPGKRSER FYECCKEPYPDVTFTVTMRRRT**GYYLIQMYIPSLLIVILSWISFWINMDAAPARVGL GITTVLTMTTQSSGSeimpatsdsvpliaqAIDIWMAVCLLFVFSALLEYAAVNFVSRQHKE LLRFRRKRRHHKSPMLNLFQEDEAGEGRFNFSAYGMGPACLQAKDGISVKGANNSN TTNPPPAPSKSPEEMRKLFIQRAKKIDKISRIGFPMAFLIFNMFYWIIYKIVRREDVHNQ (SEQ ID NO: 29, encoded by SEQ ID NO: 28); or

**(f)** *MRCSPGGVWLALAASLLHVSLQ***GEFQRKLYKELVKNYNPLERPVAND SQPLTVYFSLSLLQIMDVDEKNQVLTTNIWLQMSWTDHYLQWNVSEYPGVKTV RFPDGQIWKPDILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSSCYIDVR WFPFDVQHCKLKFGSWSYGGWSLDLQMQEADISGYIPNGEWDLVGIPGKRSER FYECCKEPYPDVTFTVTMRRRTL**YYLIQMYIPSLLIVILSWISFWINMDAAPARVGL GITTVLTMTTQSSGSeimpatsdsvpliaqAIDIWMAVCLLFVFSALLEYAAVNFVSRQHKE LLRFRRKRRHHKSPMLNLFQEDEAGEGRFNFSAYGMGPACLQAKDGISVKGANNSN TTNPPPAPSKSPEEMRKLFIQRAKKIDKISRIGFPMAFLIFNMFYWIIYKIVRREDVHNQ (SEQ ID NO:31, encoded by SEQ ID NO:30).

**[0149]** In some embodiments, the chimeric LGIC receptor is a CHRNA7/GLRA1 chimera comprising the human α7-nAChR signal peptide (italics) and ligand binding domain (bold) comprising an GlyRα1 Cys-loop sequence (lowercase); fused to the human GlyRα1 ion pore domain (underlined). In some embodiments, the chimeric LGIC receptor comprises an amino acid sequence having a sequence identity of 80% or more, 85% or more, 90% or more, 95% or more, 97% or more, 98% or more, 99% or more, or 100%, to SEQ ID NO: 33:

```
    10        20        30        40        50        60
MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL
    70        80        90       100       110       120
QIMDVDEKNQ VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV RFPDGQIWKP DILLYNSADE
    130       140       150       160       170       180
RFDATFHTNV LVNSSGHCQY LPPGIFKSSc pmdlknfpmd vqtcKLKFGS WSYGGWSLDL
    190       200       210       220       230       240
QMQEADISGY IPNGEWDLVG IPGKRSERFY ECCKEPYPDV TFTVTMRRRM GYYLIQMYIP
    250       260       270       280       290       300
SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS RASLPKVSYV KAIDIWMAVC
    310       320       330       340       350       360
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LLFVFSALLE YAAVNFVSRQ HKELLRFRRK RRHHKSPMLN LFQEDEAGEG RFNFSAYGMG
370 380 390 400 410 420
PACLQAKDGI SVKGANNSNT TNPPPAPSKS PEEMRKLFIQ RAKKIDKISR IGFPMAFLIF
430 439
NMFYWIIYKI VRREDVHNQ

(SEQ ID NO: 33, encoded by SEQ ID NO: 32).

**(a)**

*MRCSPGGVWLALAASLLHVSLQ***GEFQRKLYKELVKNYNPLERPVANDSQPLTVYFS LSLLQIMDVDEKNQVLTTNIWLQMSWTDHYLQWNVSEYPGVKTVRFPDGQIW KPDILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSScpmdlknFPFDVQHCK LKFGSWSYGGWSLDLQMQEADISGYIPNGEWDLVGIPGKRSERFYECCKEPYP DVTFTVTMRRR**MGYYLIQMYIPSLLIVILSWISFWINMDAAPARVGLGITTVLTMTT QSSGSRASLPKVSYVKAIDIWMAVCLLFVFSALLEYAAVNFVSRQHKELLRFRRKRR HHKSPMLNLFQEDEAGEGRFNFSAYGMGPACLQAKDGISVKGANNSNTTNPPPAPS KSPEEMRKLFIQRAKKIDKISRIGFPMAFLIFNMFYWIIYKIVRREDVHNQ (SEQ ID NO:35, encoded by SEQ ID NO:34).

**[0150]** In some embodiments, the chimeric LGIC receptor is a CHRNA7/GLRA1 chimera comprising the human α7-nAChR signal peptide (italics) and ligand binding domain (bold) comprising an GlyRα1 β1-2 loop sequence (lowercase); fused to the human GlyRα1 ion pore domain (underlined):

**(a)** *MRCSPGGVWLALAASLLHVSLQ***GEFQRKLYKELVKNYNPLERPVAND SQPLTVYFSLSLLQIMDVDettmVLTTNIWLQMSWTDHYLQWNVSEYPGVKTVR FPDGQIWKPDILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSSCYIDVRW FPFDVQHCKLKFGSWSYGGWSLDLQMQEADISGYIPNGEWDLVGIPGKRSERF YECCKEPYPDVTFTVTMRRR**MGYYLIQMYIPSLLIVILSWISFWINMDAAPARVGL GITTVLTMTTQSSGSRASLPKVSYVKAIDIWMAVCLLFVFSALLEYAAVNFVSRQHK ELLRFRRKRRHHKSPMLNLFQEDEAGEGRFNFSAYGMGPACLQAKDGISVKGANNS NTTNPPPAPSKSPEEMRKLFIQRAKKIDKISRIGFPMAFLIFNMFYWIIYKIVRREDVHN Q (SEQ ID NO:37, encoded by SEQ ID NO:36).

**[0151]** In some embodiments, the chimeric LGIC receptor is a CHRNA7/GLRA1 chimera comprising the human α7-nAChR signal peptide (italics) and ligand binding domain (bold) comprising an GlyRα1 β1-2 loop sequence (lowercase) and Cys-loop sequence (lowercase); fused to the human GlyRα1 ion pore domain (underlined):

**(a)** *MRCSPGGVWLALAASLLHVSLQ***GEFQRKLYKELVKNYNPLERPVAND SQPLTVYFSLSLLQIMDiaettmdLTTNIWLQMSWTDHYLQWNVSEYPGVKTVRFP DGQIWKPDILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSScpmdlknfpmd vqtcKLKFGSWSYGGWSLDLQMQEADISGYIPNGEWDLVGIPGKRSERFYECCK EPYPDVTFTVTMRRR**MGYYLIQMYIPSLLIVILSWISFWINMDAAPARVGLGITTVL TMTTQSSGSRASLPKVSYVKAIDIWMAVCLLFVFSALLEYAAVNFVSRQHKELLRFR RKRRHHKSPMLNLFQEDEAGEGRFNFSAYGMGPACLQAKDGISVKGANNSNTTNPP PAPSKSPEEMRKLFIQRAKKIDKISRIGFPMAFLIFNMFYWIIYKIVRREDVHNQ (SEQ ID NO:39, encoded by SEQ ID NO:38).

**(b)** *MRCSPGGVWLALAASLLHVSLQ***GEFQRKLYKELVKNYNPLERPVAND SQPLTVYFSLSLLQIMDVDettmVLTTNIWLQMSWTDHYLQWNVSEYPGVKTVR FPDGQIWKPDILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSScpmdlknfp mdvqtcKLKFGSWSYGGWSLDLQMQEADISGYIPNGEWDLVGIPGKRSERFYEC CKEPYPDVTFTVTMRRR**MGYYLIQMYIPSLLIVILSWISFWINMDAAPARVGLGITT VLTMTTQSSGSRASLPKVSYVKAIDIWMAVCLLFVFSALLEYAAVNFVSRQHKELLR FRRKRRHHKSPMLNLFQEDEAGEGRFNFSAYGMGPACLQAKDGISVKGANNSNTTN PPPAPSKSPEEMRKLFIQRAKKIDKISRIGFPMAFLIFNMFYWIIYKIVRREDVHNQ (SEQ ID NO:41, encoded by SEQ ID NO:40).

**(c)** *MRCSPGGVWLALAASLLHVSLQ***GEFQRKLYKELVKNYNPLERPVAND SQPLTVYFSLSLLQIMDVDettmVLTTNIWLQMSWTDHYLQWNVSEYPGVKTVR FPDGQIWKPDILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSScpmdlknfp mdvqtcKLKFGSWSYGGWSLDLQMQEADISGYIPNGEWDLVGIPGKRSERFYEC CKEPYPDVTFTVTM**ERQMGYYLIQMYIPSLLIVILSWISFWINMDAAPARVGLGITT VLTMTTQSSGSRASLPKVSYVKAIDIWMAVCLLFVFSALLEYAAVNFVSRQHKELLR FRRKRRHHKSPMLNLFQEDEAGEGRFNFSAYGMGPACLQAKDGISVKGANNSNTTN PPPAPSKSPEEMRKLFIQRAKKIDKISRIGFPMAFLIFNMFYWIIYKIVRREDVHNQ (SEQ ID NO:43, encoded by SEQ ID NO:42).

**(d)** *MRCSPGGVWLALAASLLHVSLQ***GEFQRKLYKELVKNYNPLERPVAND SQPLTVYFSLSLLQIMDiaettmdLTTNIWLQMSWTDHYLQWNVSEYPGVKTVRFP DGQIWKPDILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSScpmdlknfpmd vqtcKLKFGSWSYGGWSLDLQMQEADISGYIPNGEWDLVGIPGKRSERFYECCK EPYPDVTFTVTM**ERQMGYYLIQMYIPSLLIVILSWISFWINMDAAPARVGLGITTVLT MTTQSSGSRASLPKVSYVKAIDIWMAVCLLFVFSALLEYAAVNFVSRQHKELLRFRR

KRRHHKSPMLNLFQEDEAGEGRFNFSAYGMGPACLQAKDGISVKGANNSNTTNPPP APSKSPEEMRKLFIQRAKKIDKISRIGFPMAFLIFNMFYWIIYKIVRREDVHNQ (SEQ ID NO:45, encoded by SEQ ID NO:44).

**[0152]** In some embodiments, the chimeric LGIC receptor is a CHRNA7/GLRA1 chimera comprising the human α7-nAChR signal peptide (italics) and ligand binding domain (bold) comprising an GlyRα1 β1-2 loop sequence (lowercase); fused to the human GlyRα1 ion pore domain (underlined) comprising human α7-nAChR M2-M3 linker (lowercase):

**(a)** *MRCSPGGVWLALAASLLHVSLQ***GEFQRKLYKELVKNYNPLERPVAND SQPLTVYFSLSLLQIMDVDettmVLTTNIWLQMSWTDHYLQWNVSEYPGVKTVR FPDGQIWKPDILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSSCYIDVRW FPFDVQHCKLKFGSWSYGGWSLDLQMQEADISGYIPNGEWDLVGIPGKRSERF YECCKEPYPDVTFTVTMRRR**MGYYLIQMYIPSLLIVILSWISFWINMDAAPARVGL GITTVLTMTTQSSGSeimpatsdsvpliaqAIDIWMAVCLLFVFSALLEYAAVNFVSRQHKE LLRFRRKRRHHKSPMLNLFQEDEAGEGRFNFSAYGMGPACLQAKDGISVKGANNSN TTNPPPAPSKSPEEMRKLFIQRAKKIDKISRIGFPMAFLIFNMFYWIIYKIVRREDVHNQ (SEQ ID NO:47, encoded by SEQ ID NO:46).

**[0153]** In some embodiments, the chimeric LGIC receptor is a CHRNA7/GLRA1 chimera comprising the human α7-nAChR signal peptide (italics) and ligand binding domain (bold) comprising a GlyRα1 Cys-loop sequence (lowercase); fused to the human GlyRα1 ion pore domain (underlined) comprising a human α7-nAChR M2-M3 linker (lowercase):

**(a)** *MRCSPGGVWLALAASLLHVSLQ***GEFQRKLYKELVKNYNPLERPVAND SQPLTVYFSLSLLQIMDVDEKNQVLTTNIWLQMSWTDHYLQWNVSEYPGVKTV RFPDGQIWKPDILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSScpmdlknf pmdvqtcKLKFGSWSYGGWSLDLQMQEADISGYIPNGEWDLVGIPGKRSERFYEC CKEPYPDVTFTVTMRRR**MGYYLIQMYIPSLLIVILSWISFWINMDAAPARVGLGITT VLTMTTQSSGSeimpatsdsvpliaqAIDIWMAVCLLFVFSALLEYAAVNFVSRQHKELLRF RRKRRHHKSPMLNLFQEDEAGEGRFNFSAYGMGPACLQAKDGISVKGANNSNTTNP PPAPSKSPEEMRKLFIQRAKKIDKISRIGFPMAFLIFNMFYWIIYKIVRREDVHNQ (SEQ ID NO:49, encoded by SEQ ID NO:48).

**[0154]** In some embodiments, the chimeric LGIC receptor is a HTR3A/GLRA1 chimera (R241 junction), comprising the human 5HT3A serotonin receptor signal peptide (italics) and ligand binding domain (bold), fused to the human GlyRα1 ion pore domain (underlined):

**(a)** *MLLWVQQALLALLLPTLLAQGEA***RRSRNTTRPALLRLSDYLLTNYRK GVRPVRDWRKPTTVSIDVIVYAILNVDEKNQVLTTYIWYRQYWTDEFLQWNPE DFDNITKLSIPTDSIWVPDILINEFVDVGKSPNIPYVYIRHQGEVQNYKPLQVVTA CSLDIYNFPFDVQNCSLTFTSWLHTIQDINISLWRLPEKVKSDRSVFMNQGEWEL LGVLPYFREFSMESSNYYAEMKFYVVIRRR**MGYYLIQMYIPSLLIVILSWISFWINM DAAPARVGLGITTVLTMTTQSSGSRASLPKVSYVKAIDIWMAVCLLFVFSALLEYAA VNFVSRQHKELLRFRRKRRHHKSPMLNLFQEDEAGEGRFNFSAYGMGPACLQAKD GISVKGANNSNTTNPPPAPSKSPEEMRKLFIQRAKKIDKISRIGFPMAFLIFNMFYWIIY KIVRREDVHNQ (SEQ ID NO:50).

**[0155]** In some embodiments, the chimeric LGIC receptor is a HTR3A/GLRA1 chimera (V236 junction) comprising the human 5HT3A serotonin receptor signal peptide (italics) and ligand binding domain (bold), fused to the human GlyRα1 ion pore domain (underlined):

**(a)** *MLLWVQQALLALLLPTLLAQGEA***RRSRNTTRPALLRLSDYLLTNYRK GVRPVRDWRKPTTVSIDVIVYAILNVDEKNQVLTTYIWYRQYWTDEFLQWNPE DFDNITKLSIPTDSIWVPDILINEFVDVGKSPNIPYVYIRHQGEVQNYKPLQVVTA CSLDIYNFPFDVQNCSLTFTSWLHTIQDINISLWRLPEKVKSDRSVFMNQGEWEL LGVLPYFREFSMESSNYYAEMKFYV**HLERQMGYYLIQMYIPSLLIVILSWISFWINM DAAPARVGLGITTVLTMTTQSSGSRASLPKVSYVKAIDIWMAVCLLFVFSALLEYAA VNFVSRQHKELLRFRRKRRHHKSPMLNLFQEDEAGEGRFNFSAYGMGPACLQAKD GISVKGANNSNTTNPPPAPSKSPEEMRKLFIQRAKKIDKISRIGFPMAFLIFNMFYWIIY KIVRREDVHNQ (SEQ ID NO:51).

**[0156]** In some embodiments, the chimeric LGIC receptor is a GABRB3/GLRA1 chimera (Y245 junction), comprising the human GABA-A β3 signal peptide (italics) and ligand binding domain (bold), fused to the human GlyRα1 ion pore domain (underlined):

**(a)** *MWGLAGGRLFGIFSAPVLVAVVCCA***QSVNDPGNMSFVKETVDKLLKG YDIRLRPDFGGPPVCVGMNIDIASIDMVSEVNMDYTLTMYFQQYWRDKRLAYS GIPLNLTLDNRVADQLWVPDTYFLNDKKSFVHGVTVKNRMIRLHPDGTVLYGL RITTTAACMMDLRRYPLDEQNCTLEIESYGYTTDDIEFYWRGGDKAVTGVERIE LPQFSIVEHRLVSRNVVFATGAYPRLSLSFRLKRNIGY**MGYYLIQMYIPSLLIVILS WISFWINMDAAPARVGLGITTVLTMTTQSSGSRASLPKVSYVKAIDIWMAVCLLFVF SALLEYAAVNFVSRQHKELLRFRRKRRHHKSPMLNLFQEDEAGEGRFNFSAYGMGP

ACLQAKDGISVKGANNSNTTNPPPAPSKSPEEMRKLFIQRAKKIDKISRIGFPMAFLIF NMFYWIIYKIVRREDVHNQ (SEQ ID NO:52).

### *C1. Amino Acid Mutations*

**[0157]** As discussed above, in some aspects, the subject engineered receptor comprises at least one amino acid mutation that alters the potency of a ligand on the engineered receptor relative to its potency on the unmutated parental receptor. Put another way, the one or more amino acid mutations, *e.g.* a loss-of-function mutations or a gain-of-function mutations, shift the potency of the engineered receptor to the ligand relative to the potency of the unmutated parental receptor. In some embodiments, the mutation is an amino acid substitution. In some embodiments, the one or more mutations is in the ligand binding domain of the engineered receptor. In some embodiments, as when the ligand binding domain of the engineered receptor is a Cys-loop receptor protein, the one or more amino acid mutations is a substitution at a residue corresponding to a residue of α7-nAChR (SEQ ID NO:4) selected from the group consisting of W77, Y94, R101, W108, Y115, T128, N129, V130, L131, Q139, L141, Y151, S170, W171, S172, S188, Y190, Y210, C212, C213 and Y217. In some embodiments, one residue is substituted. In some embodiments, 2, 3, 4, or 5 or more residues are substituted, *e.g.* 6, 7, 8, 9 or 10 residues are substituted. In certain embodiments, the residue corresponds to a residue of α7-nAChR (SEQ ID NO:4) that is selected from the group consisting of W77, R101, Y115, N129, L131, S170, S172, and S188. In certain embodiments, the one or more substitutions is within an α7-nAChR sequence.

**[0158]** In some embodiments, the one or more substitutions decreases, *e.g.* 2-fold or more, 3-fold or more, 4-fold or more. 5-fold or more, 10-fold or more, 20-fold or more, 30-fold or more, 50-fold or more, or 100-fold, the potency of an engineered receptor to acetylcholine and a non-native ligand. In certain embodiments, the one or more substitutions is a substitution corresponding to R101I, R101S, R101D, Y115L, Y115M, Y115D, Y115T, T128M, T128R, T128I, N129I, N129V, N129P, N129W, N129T, N129D, N129E, L131E, L131P, L131T, L131D, L131S, L141S, L141R, W171F, W171H, S172F, S172Y, S172R, S172D, C212A, C212L, or C213P of α7-nAChR. In other instances, the one or more substitutions decreases the potency of acetylcholine on the engineered receptor selectively. In other words, the one or more substitutions decreases the potency of the engineered receptor to acetylcholine while essentially maintaining potency to non-native ligand or otherwise decreasing the potency of the engineered receptor to acetylcholine 2-fold or more, *e.g.* 3-fold, 4-fold, 5-fold or more, in some instances 10-fold, 20-fold, 50-fold, or 100-fold or more, than it

decreases the potency of the engineered receptor to non-native ligand. In some embodiments, the substitution corresponds to L131E, L131S, L131T, L131D, or S172D of α7-nAChR. In yet other embodiments, the one or more substitutions decreases the potency of a non-native ligand on the engineered receptor selectively. In other words, the one or more substitutions decreases the potency of the engineered receptor to non-native ligand while essentially maintaining potency to acetylcholine or otherwise decreasing the potency of the engineered receptor to non-native ligand 2-fold or more, *e.g.* 3-fold, 5-fold or more, in some instances 10-fold, 20-fold or 50-fold or more, than it decreases the potency of the engineered receptor to acetylcholine. In some embodiments, the substitution corresponds to W77M, Y115W, S172T, or S172C of α7-nAChR. In certain embodiments, the one or more substitutions is within an α7-nAChR sequence. In certain embodiments, the non-native ligand is selected from AZD-0328, TC6987, ABT-126, and Facinicline/RG3487.

**[0159]** In other embodiments, the one or more substitutions increases, *e.g.* 2-fold or more, 3-fold or more, 4-fold or more. 5-fold or more, 10-fold or more, 20-fold or more, 30-fold or more, 50-fold or more, or 100-fold, the potency of the engineered receptor to acetylcholine and/or non-native ligand. In some embodiments, the substitution corresponds to L131N, L141W, S170G, S170A, S170L, S170I, S170V, S170P, S170F, S170M, S170T, S170C, S172T, S172C, S188I, S188V, S188F, S188M, S188Q, S188T, S188P or S188W. In some embodiments. the one or more substitutions increases potency of both acetylcholine and non-native ligand. In some embodiments, the substitution corresponds to L131N, S170G, S170A, S170L, S170I, S170V, S170P, S170F, S170M, S170T, S170C, S172T, S188I, S188V, S188F, S188M, S188Q or S188T of α7-nAChR. In other instances, the one or more substitutions increases the potency of acetylcholine on the engineered receptor selectively. In other words, the one or more substitutions increases the potency of the engineered receptor to acetylcholine 2-fold or more, *e.g.* 3-fold, 4-fold, or 5-fold or more, in some instances 10-fold, 20-fold, 50-fold, or 100-fold, than it increases the potency of the engineered receptor to non-native ligand. In some embodiments, the substitution corresponds to L141W, S172T, S172C, S188P or S188W, of α7-nAChR. In certain embodiments, the one or more substitutions is within an α7-nAChR sequence. In certain embodiments, the non-native ligand is selected from AZD-0328, TC6987, ABT-126 and Facinicline/RG3487. In yet other instances, the one or more substitutions increases the potency of the non-native ligand on the engineered receptor selectively. In other words, the one or more substitutions increases the potency of the engineered receptor to non-native ligand 2-fold or more, *e.g.* 3-fold, 5-fold or more, in some

instances 10-fold, 20-fold or 50-fold or more, than it increases the potency of the engineered receptor to acetylcholine.

**[0160]** In some embodiments, the amino acid residue that is mutated in the subject engineered receptor is not an amino acid corresponding to R27, E41, Q79, Q139, L141, G175, Y210, P216, Y217, or D219 of wild type a7 nAChR (SEQ ID NO:4). In some embodiments, the mutation is an amino acid substitution. In some embodiments, the amino acid residue that is mutated in the subject engineered receptor is an amino acid corresponding to R27, E41, Q79, Q139, L141, G175, Y210, P216, Y217, or D219 of wild type a7 nAChR (SEQ ID NO:4). In some embodiments, the substitution is not a substitution corresponding to W77F, W77Y, W77M, Q79A, Q79Q, Q79S, Q79G, Y115F, L131A, L131G, L131M, L131N, L131Q, L131V, L131F, Q139G, Q139L, G175K, G175A, G175F, G175H, G175M, G175R, G175S, G175V, Y210F, P216I, Y217F, or D219A in wild type α7 nAChR. In some embodiments, the substitution is a substitution corresponding to W77F, W77Y, W77M, Q79A, Q79Q, Q79S, Q79G, Y115F, L131A, L131G, L131M, L131N, L131Q, L131V, L131F, Q139G, Q139L, G175K, G175A, G175F, G175H, G175M, G175R, G175S, G175V, Y210F, P216I, Y217F, or D219A in wild type α7 nAChR. In some embodiments, when such a substitution exists within the engineered receptor, it exists in combination with one or more of the amino acid mutations described herein.

**[0161]** In some embodiments, residues Y94, Y115, Y151, and Y190 of α7-nAChR (SEQ ID NO:4) mediate binding of the native ligand acetylcholine. In some embodiments, mutations at these residues may reduce binding of acetylcholine and hence be considered loss of function mutations. In some embodiments, residues W77, Y115, N129, V130, L131, Q139, L141, S170, Y210, C212, C213 and Y217 of the α7-nAChR may mediate the binding of non-native ligand AZD0328 to this receptor, and mutation of these residues may increase the affinity of AZD0328 and/or other ligands for this receptor and hence be considered gain-of-function mutations. In some embodiments, the subject engineered receptor comprises a mutation in one or more amino acid residues of the ligand binding domain region of α7-nAChR (SEQ ID NO:4) or the ligand binding domain of a chimeric receptor that comprises the ligand binding domain region of α7-nAChR, wherein the one or more amino acid residues is selected from the group consisting of W77, Y94, Y115, N129, V130, L131, Q139, L141, Y151, S170, Y190, Y210, C212, C213 and Y217. In some embodiments, the mutation is an amino acid substitution. In certain embodiments, the mutation in the one or more amino acid residues of the ligand binding domain region of α7-nAChR (SEQ ID NO:4) or the ligand binding domain of a chimeric receptor that comprises the ligand binding domain region of α7-nAChR is a

substitution at one or more amino acid residues selected from the group consisting of W77, Y94, Y115, N129, V130, L131, Q139, L141, Y151, S170, Y190, Y210, C212, C213 and Y217.

**[0162]** In some embodiments, residues Y115, L131, L141, S170, W171, S172, C212, and Y217 of α7-nAChR (SEQ ID NO:4) may mediate binding of acetylcholine and/or nicotine, and mutations at one or more of these residues may reduce binding of acetylcholine and/or nicotine. In some embodiments, R101, Y115, L131, L141, W171, S172, S188, Y210, and Y217 of α7-nAChR may mediate binding of the non-native ligand ABT126, and mutation of one or more of these residues may increase the affinity of ABT126 and/or other ligands for α7-nAChR. In some embodiments, the mutation is an amino acid substitution. In some embodiments, R101, Y115, T128, N129, L131, L141, W171, S172, Y210, C212, C213 and Y217 of α7-nAChR may mediate binding of the non-native ligand TC6987, and mutation of one or more of these residues may increase the affinity of TC6987 and/or other ligands for α7-nAChR. In some embodiments, R101, N120, L131, L141, S170, W171, S172, Y210, and Y217 of α7-nAChR may mediate binding of the non-native ligand Facinicline/RG3487, and mutation of one or more of these residues may increase the affinity of Facinicline/RG3487and/or other ligands for α7-nAChR. In some embodiments, the subject engineered receptor comprises a mutation in one or more amino acid residues of the ligand binding domain region of α7-nAChR or the ligand binding domain of a chimeric receptor that comprises the ligand binding domain region of α7-nAChR, where the one or more amino acid residues is selected from the group consisting of R101, Y115, T128, N120, N129, L131, L141, S170, W171, S172, S188, Y210, C212, C213 and Y217. In some embodiments, the one or more amino acid residues alters the binding of acetylcholine and/or nicotine to α7-nAChR, wherein the amino acid is selected from the group consisting of Y115, L131, L141, S170, W171, S172, C212 and Y217 of α7-nAChR. In certain such embodiments, the amino acid is selected from C212 and S170. In some embodiments, the mutation in the one or more amino acid residues alters the binding of ABT126 to α7-nAChR, wherein one or more amino acid residues is selected from the group consisting of R101, Y115, L131, L141, W171, S172, S188, Y210, and Y217 of α7-nAChR. In certain such embodiments, the amino acid is selected from R101, S188, and Y210. In some embodiments, the mutation in the one or more amino acid residues alters the binding of TC6987 to α7-nAChR, wherein one or more amino acid residues is selected from the group consisting of R101, Y115, T128, N129, L131, L141, W171, S172, Y210, C212, C213 and Y217 of α7-nAChR. In certain such embodiments, the amino acid is selected from R101, T128, N129, Y210 and C213. In some embodiments, the mutation in the one or more amino acid residues alters the binding of Facinicline/RG3487 to α7-nAChR, wherein one or more amino acid

residues is selected from the group consisting of R101, N120, L131, L141, S170, W171, S172, Y210, and Y217 of α7-nAChR. In certain such embodiments, the amino acid is selected from Y210, R101, and N129.

**[0163]** In some embodiments, residues W85, R87, Y136, Y138, G146, N147, Y148, K149, S177, S178, L179, Y228, and Y229 of 5HT3 (SEQ ID NO:6) may mediate binding of serotonin, and mutations at one or more of these residues may reduce binding of serotonin to 5HT3. D64, I66, W85, R87, Y89, N123, G146, Y148, T176, S177, S178, W190, R191, F221, E224, Y228, Y229 and E231 of 5HT3 mediate binding of the non-native ligand Cilansetron, and mutation of one or more of these residues may increase the affinity of Cilansetron and/or other ligands for 5HT3. In some embodiments, the mutation is an amino acid substitution. In some embodiments, the subject engineered receptor comprises a mutation in one or more amino acid residues of the ligand binding domain region 5HT3A or the ligand binding domain of a chimeric receptor that comprises the ligand binding domain region of 5HT3, where the one or more amino acid residues is selected from the group consisting of D64, I66, W85, R87, Y89, N123, Y136, Y138, G146, N147, Y148, K149, T176, S177, S178, L179, W190, R191, F221, E224, Y228, Y229, and E231. In some embodiments, the mutation in the one or more amino acid residues alters the binding of serotonin to 5HT3, wherein the amino acid is selected from the group consisting of W85, R87, Y136, Y138, G146, N147, Y148, K149, S177, S178, L179, Y228, and Y229 of 5HT3A. In certain such embodiments, the amino acid is selected from Y136, Y138, N147, K149, and L179. In some embodiments, the mutation in the one or more amino acid residues alters the binding of Cilansetron to 5HT3 wherein one or more amino acid residues is selected from the group consisting of D64, I66, W85, R87, Y89, N123, G146, Y148, T176, S177, S178, W190, R191, F221, E224, Y228, Y229 and E231 of 5HT3A. In certain such embodiments, the amino acid is selected from D64, I66, Y89, N123, T176, W190, R191, F221, E224, and E231.

**[0164]** In some embodiment, the one or more mutations that affects the ability of a ligand to modulate the activity of the LGIC is located in the ion pore domain of the LGIC. In some embodiments, the mutation is an amino acid substitution. For example, residue T279 of the serotonin receptor 5HT3A mediates the way in which the ligand modulates the activity of the channel, such that mutation of this residue to, *e.g.* serine (T279S), converts the effect from being antagonistic (i.e., reducing the activity of the LGIC) to agonistic (i.e. promoting the activity of the channel). In some embodiments, the subject ligand gated ion channel comprises a mutation in one or more amino acid residues of the ion pore domain of the human 5HT3A (SEQ ID NO:6) or the ion pore domain of a chimeric LGIC receptor that comprises the ion

pore domain of 5HT3A, where the substitution is in an amino acid corresponding to 279 of SEQ ID NO:6. In certain embodiments, the substitution is a T279S substitution relative to SEQ ID NO:6.

**[0165]** The disclosure provides engineered receptors having two or more mutations, such as amino acid substitutions, as compared to the parental receptor. In some embodiments, the parental receptor comprises a ligand binding domain derived from human α7 nicotinic acetylcholine receptor (α7-nAChR). In some embodiments, the parental receptor is a chimeric receptor. In some embodiments, the parental receptor comprises an ion pore domain derived from a human Glycine receptor. In some embodiments, the human Glycine receptor is human Glycine receptor α1, human Glycine receptor α2, or human Glycine receptor α3. In some embodiments, the ligand binding domain of the engineered receptor comprises a Cys-loop domain derived from the human Glycine receptor. In some embodiments, the parental receptor comprises an amino acid sequence of SEQ ID NO: 33. In some embodiments, the engineered receptors comprise two amino acid substitutions as compared to the parental receptor comprising an amino acid sequence of SEQ ID NO: 33. In some embodiments, the ligand binding domain of the engineered receptor comprises a β1-2 loop domain from the human Glycine receptor α1 subunit.

**[0166]** In some embodiments, the ligand binding domain of the engineered receptor comprises amino acid substitutions at two or more amino acid residues selected from those corresponding to W77, R101, Y115, L131, Q139, Y140, S170, S172, Y210, and Y217 of human α7-nAChR (SEQ ID NO: 4).

**[0167]** In some embodiments, the two amino acid substitutions are at a pair of amino acid residues selected from the group consisting of L131 and S172, Y115 and S170, Y115 and S172, and Y115 and L131. In some embodiments, the ligand binding domain comprises two amino acid substitutions at a pair of amino acids residues selected from the group consisting of L131 and S172, Y115 and S170, Y115 and S172, and Y115 and L131. In some embodiments, the ligand binding domain comprises an amino acid substitution at residue L131 and the amino acid substitution of S172D. In some embodiments, the ligand binding domain comprises an amino acid substitution at residue L131 and the amino acid substitution of Y115D. In some embodiments, the ligand binding domain comprises a pair of amino acid substitutions selected from the group consisting of L131S and S172D, L131T and S172D, L131D and S172D, Y115D and S170T, Y115D and L131Q, Y115Q and S172D, Y115E and L131M, and Y115D and L131E. In some embodiments, the ligand binding domain comprises an amino acid substitution of L131E.

**[0168]** In some embodiments, the ligand binding domain comprises one or more amino acid substitutions at amino acids residues selected from the group consisting of Y140, R101, L131, Y115, and Y210, wherein the amino acid residues correspond to the amino acid residues of α7-nAChR. In some embodiments, the ligand binding domain comprises an amino acid substitution of R101W and/or Y210V. In some embodiments, the ligand binding domain comprises two or more amino acid substitutions at amino acid residues selected from the group consisting of R101, L131, Y115, Y210, and Y140. In some embodiments, the ligand binding domain comprises two amino acid substitutions at amino acid residues selected from the group consisting of R101, L131, Y115, Y210, and Y140. In some embodiments, the ligand binding domain comprises two amino acid substitutions at a pair of amino acid residues selected from the group consisting of: R101 and L131, Y115 and Y210, R101 and Y210. In some embodiments, the ligand binding domain comprises a pair of amino acid substitutions selected from the group consisting of R101F and L131G, R101F and L131D, Y115E and Y210W, R101W and Y210V, R101F and Y210V, R101F and Y210F, R101M and L131A, and R101M and L131F. In some embodiments, the ligand binding domain comprises three amino acid substitutions at the amino acid residues R101, Y115, and Y210. In some embodiments, the ligand binding domain comprises amino acid substitutions R101W, Y115E, and Y210W, or the amino acid substitutions R101F, Y115E, and Y210W.

**[0169]** In some embodiments, the ligand binding domain comprises an amino acid substitution at residue L131 and the amino acid substitution of R101F or R101M. In some embodiments, the amino acid substitution at residue L131 is L131G, L131D, L131A, L131F, or L131N.

**[0170]** In some embodiments, the ligand binding domain comprises a hydrophobic amino acid substitution at residue Y210 and the amino acid substitution of R101W or R101F. In some embodiments, the amino acid substitution at residue Y210 is Y210V, Y210F, or Y210W.

**[0171]** In some embodiments, the ligand binding domain comprises an amino acid substitution of Q139W and an additional amino acid substitution at a residue selected from S172, Y210 and Y217.

**[0172]** A person skilled in the art will readily recognize proper control receptor for comparison with the engineered receptor of the disclosure. In some embodiments, the control receptor is identical in sequence to the engineered receptor except for the one or more distinguishing amino acid mutations (e.g., substitutions). In all cases, references to a control

receptor is meant to indicate that the recited change of property (e.g., potency to a ligand) is the result of the amino acid mutation(s) of the engineered receptor of the disclosure.

**[0173]** The disclosure provides engineered receptors, wherein the engineered receptor is a chimeric ligand gated ion channel (LGIC) receptor and comprises: (a) a ligand binding domain derived from the human α7 nicotinic acetylcholine receptor (α7-nAChR) and comprising a Cys-loop domain from the human Glycine receptor α1 subunit; and (b) an ion pore domain derived from the human Glycine receptor α1 subunit. In some embodiments, the engineered receptor is derived from a parental engineered receptor comprising or consisting of an amino acid sequence of SEQ ID NO: 33, and further comprises one or more amino acid substitutions based on the parental engineered receptor.

**[0174]** In some embodiments, the potency of the engineered receptor to acetylcholine is lower than the potency of the human α7 nicotinic acetylcholine receptor (α7-nAChR) to acetylcholine. In some embodiments, the potency of the engineered receptor to acetylcholine is at least about 1.5-fold (for example, about 2-fold lower, about 3-fold, about 4-fold, about 5-fold, about 6-fold, about 7-fold, about 8-fold, about 9-fold, about 10-fold, about 12-fold, about 15-fold, about 20-fold, about 30-fold, about 40-fold, about 50-fold, about 60-fold, about 70-fold, about 80-fold, about 90-fold, or about 100-fold, including all subranges and values that lie therebetween) lower than the potency of the human α7 nicotinic acetylcholine receptor (α7-nAChR) to acetylcholine. In some embodiments, the potency of the engineered receptor to acetylcholine is evaluated by its EC50 for acetylcholine based on a cell reporter assay using YFP fluorescence quenching as described in Example 2 of the disclosure. In some embodiments, the EC50 of the engineered receptor to acetylcholine is at least 100 uM, at least 200 uM, at least 300 uM, at least 500 uM, at least 700 uM, at least 1 mM, at least 2 mM, at least 3 mM, at least 4 mM, at least 5 mM, at least 6 mM, at least 7 mM, at least 8 mM, at least 9 mM, or at least 10 mM. In some embodiments, the EC50 of the engineered receptor to acetylcholine is at least 1 mM. In some embodiments, the EC50 of the engineered receptor to acetylcholine is at least 3 mM. In some embodiments, having a higher EC50 for acetylcholine permits higher expression level of the engineered receptor in the cell or on the cell surface, without passing significant amount of current into the cell at the presence of physiological concentration of acetylcholine.

**[0175]** In some embodiments, the potency of the engineered receptor to a non-native ligand is about the same as the potency of the human α7 nicotinic acetylcholine receptor (α7-nAChR) to the non-native ligand. In some embodiments, the potency of the engineered receptor to a non-native ligand is higher than the potency of the human α7 nicotinic acetylcholine

receptor (α7-nAChR) to the non-native ligand. In some embodiments, the potency of the engineered receptor to the non-native ligand is at least about 1.5-fold (for example, about 2-fold lower, about 3-fold, about 4-fold, about 5-fold, about 6-fold, about 7-fold, about 8-fold, about 9-fold, about 10-fold, about 12-fold, about 15-fold, about 20-fold, about 30-fold, about 40-fold, about 50-fold, about 60-fold, about 70-fold, about 80-fold, about 90-fold, or about 100-fold, including all subranges and values that lie therebetween) higher than the potency of the human α7 nicotinic acetylcholine receptor (α7-nAChR) to the non-native ligand. In some embodiments, determining the potency comprises determining the EC50 based on a cell reporter assay using YFP fluorescence quenching as described in Example 2 of the disclosure. In some embodiments, the EC50 of the engineered receptor to a non-native ligand is less than 1 nM, less than 2 nM, less than 3 nM, less than 4 nM, less than 5 nM, less than 6 nM, less than 7 nM, less than 8 nM, less than 9 nM, less than 10 nM, less than 15 nM, less than 20 nM, less than 30 nM, less than 40 nM, less than 50 nM, less than 60 nM, less than 70 nM, less than 80 nM, less than 90 nM, less than 100 nM, less than 150 nM, less than 200 nM, less than 300 nM, less than 400 nM, less than 500 nM, less than 600 nM, less than 700 nM, less than 800 nM, less than 900 nM, less than 1 uM, less than 2 uM, less than 3 uM, less than 4 uM, less than 5 uM, less than 6 uM, less than 7 uM, less than 8 uM, less than 9 uM, or less than 10 uM. In some embodiments, the EC50 of the engineered receptor to a non-native ligand is less than 10 nM. In some embodiments, the EC50 of the engineered receptor to a non-native ligand is less than 100 nM. In some embodiments, the EC50 of the engineered receptor to a non-native ligand is less than 1 uM.

**[0176]** In some embodiments, the efficacy of the engineered receptor in the presence of a non-native ligand is higher than the efficacy the human α7 nicotinic acetylcholine receptor (α7-nAChR) in presence of the non-native ligand. In some embodiments, the efficacy of the engineered receptor in the presence of a non-native ligand is at least about 1.5-fold (for example, about 2-fold lower, about 3-fold, about 4-fold, about 5-fold, about 6-fold, about 7-fold, about 8-fold, about 9-fold, about 10-fold, about 12-fold, about 15-fold, about 20-fold, about 30-fold, about 40-fold, about 50-fold, about 60-fold, about 70-fold, about 80-fold, about 90-fold, or about 100-fold, including all subranges and values that lie therebetween) higher than the efficacy the human α7 nicotinic acetylcholine receptor (α7-nAChR) in presence of the non-native ligand. In some embodiments, determining the efficacy comprises determining the amount of current passed through the engineered receptor *in vitro* in the presence of the non-native ligand.

**[0177]** In some aspects, the subject ligand-gated ion channel comprises one or more non-desensitizing mutations. In some embodiments, the mutation is an amino acid substitution. When used in the context of a ligand-gated ion channel, "desensitization" refers to the progressive reduction in ionic flux in the prolonged presence of agonist. This results in a progressive loss of potency of the neuron to the ligand. By a non-desensitizing mutation, it is meant an amino acid mutation that prevents the LGIC from becoming desensitized to ligand, thereby preventing the neuron from becoming less responsive or nonresponsive to ligand. Non-desensitizing mutations can be readily identified by introducing the LGIC carrying the mutation into a neuron and analyzing the current flux over time during prolonged exposure to ligand. If the LGIC does not comprise a non-desensitizing mutation, the current will restore from peak to steady state during prolonged exposure, whereas if the LGIC comprises a non-desensitizing mutation, the current will remain at peak flux for the duration of exposure to ligand. Exemplary amino acid mutations that result in desensitization include a V322L mutation in the human GlyRα1 (V294L post-processing of the pro-protein to remove the signal peptide) and a L321V mutation in human GABA-A receptor GABRB3 (L296V post-processing of the pro-protein to remove the signal peptide). In some embodiments, the desensitizing mutation is the replacement of amino acid residues at or near the C-terminus of the LGIC with a desensitizing sequence, for example, a sequence having 90% identity or more to IDRLSRIAFPLLFGIFNLVYWATYLNREPQL (SEQ ID NO:53) derived from the C terminus of the protein encoded by GABAR1, *e.g.* the replacement of residues 455-479 in GABRR1 with IDRLSRIAFPLLFGIFNLVYWATYLNREPQL (SEQ ID NO:53). LGIC desensitization, methods for measuring desensitization of LGICs, and mutations that are non-desensitizing are well known in the art; see, *e.g.* Gielen et al. Nat Commun 2015 Apr 20, 6:6829, and Keramidas et al. Cell Mol Life Sci. 2013 Apr;70(7):1241-53, the full disclosures of which are incorporated herein by reference.

**[0178]** In some aspects, the subject ligand-gated ion channel comprises one or more conversion mutations. In some embodiments, the mutation is an amino acid substitution. By a conversion mutation, it is meant a mutation that changes the permeability of the ion pore domain of the LGIC such that it becomes permissive to the conductance of a non-native ion, i.e. an ion that does not naturally allow to pass through. In some cases, the mutation converts the permeability from cation to anion, for example the replacement of amino acid residues 260-281 in human α7-nAChR (CHRNA7) (EKISLGITVLLSLTVFMLLVAE, SEQ ID NO:54) or the corresponding amino acids in another cation-permeable LGIC with the peptide sequence PAKIGLGITVLLSLTTFMSGVAN (SEQ ID NO:55). In some cases, the mutation converts

the permeability from anion to cation, for example, the substitution of amino acid residue 279 of GLRA1 or the corresponding amino acid in another anion-permeable LGIC to glutamic acid (E), (which, as an A293E substitution in GLRA1 converts the LGIC from being anion-permissive to calcium-permissive), or the deletion of amino acid residue 278 of GLRA1 or the corresponding amino acid in another anion-permeable LGIC, the substitution of amino acid residue 279 of GLRA1 or the corresponding amino acid in another anion-permeable LGIC to glutamic acid (E), and the substitution of amino acid residue 293 of GLRA1 or the corresponding amino acid in another anion-permeable LGIC to valine (V) (which, as a P278Δ, A279E, T293V in GLRA1 converts the LGIC from being anion-permissive to cation-permissive).

**[0179]** Additional engineered receptors beyond those described herein can be readily identified by *in vitro* screening and validation methods. In some embodiments, a library of parental receptor mutants is generated from a limited number of parental receptors. The parental receptors can be mutated using methods known in the art, including error prone PCR. In some embodiments, the library of parental receptor mutants is then transfected into yeast or mammalian cells and screened in high throughput to identify functional receptors (*e.g.*, to identify parental receptor mutants that are capable of signaling in response to a ligand). In some embodiments, the functional parental receptor mutants identified in this primary screen is then expressed in mammalian cells and screened for potency to ligands, *e.g.* by the plate reader and/or electrophysiology assays described herein. The parental receptor mutants that demonstrate either increased binding affinity for agonist ligands, or that enable the use of antagonist or modulator ligands as agonists in the secondary screen can then be selected and carried though further *in vitro* and/or *in vivo* validation and characterization assays. Such screening assays are known in the art, for example Armbruster, B.N. *et al.* (2007) PNAS, 104, 5163–5168; Nichols, C.D. and Roth, B.L. (2009) Front. Mol. Neurosci. 2, 16; Dong, S. *et al.* (2010) Nat. Protoc. 5, 561–573; Alexander, G.M. *et al.* (2009) Neuron 63, 27–39; Guettier, J.M. *et al.* (2009) PNAS 106, 19197–19202; Ellefson J.W. *et al.* (2014) Nat Biotechnol. 32(1):97-101; Maranhao AC and Ellington AD. (2017) ACS Synth Biol. 20;6(1):108-119; Talwar S *et al.* (2013) PLoS One;8(3):e58479; Gilbert D.F. *et al.* (2009) Front Mol Neurosci. 30;2:17; Lynagh and Lynch, (2010), Biol Chem. 14:285(20), 14890–14897; Islam R. *et al.* (2016) ACS Chem Neurosci. 21;7(12):1647-1657; and Myers *et al.* (2008) Neuron. 8:58(3): 362–373.

*C2. Exemplary chimeric LGICs*

**[0180]** In some embodiments, the engineered receptor comprises a ligand binding domain derived from human α7-nAChR and the amino acid substitutions corresponding to L131S and S172D in the LBD of human α7-nAChR. In some embodiments, such an engineered receptor has a lower potency (*e.g.*, as determined by a higher EC50) for acetylcholine as compared to a control receptor without the substitutions or with only one of such substitutions. In some embodiments, such an engineered receptor substantially retains the potency (or has a higher potency) for a non-native ligand as compared to a control receptor without such substitutions or with only one of such substitutions. In some embodiments, the non-native ligand is CNL001. In some embodiments, the engineered receptor is a chimeric LGIC comprising a ligand binding domain derived from human α7-nAChR and an ion pore domain derived from human GlyRα1. In some embodiments, the engineered receptor is CODA534 (SEQ ID NO: 59), and the control receptor is CODA71, CODA333, or CODA377.

**[0181]** In some embodiments, the engineered receptor comprises a ligand binding domain derived from human α7-nAChR and the amino acid substitutions corresponding to L131T and S172D in the LBD of human α7-nAChR. In some embodiments, such an engineered receptor has a lower potency (*e.g.*, as determined by a higher EC50) for acetylcholine as compared to a control receptor without the substitutions or with only one of such substitutions. In some embodiments, such an engineered receptor has an EC50 for acetylcholine of >3 mM. In some embodiments, such an engineered receptor substantially retains the potency (or has a higher potency) for a non-native ligand as compared to a control receptor without such substitutions or with only one of such substitutions. In some embodiments, the non-native ligand is CNL001. In some embodiments, the engineered receptor is a chimeric LGIC comprising a ligand binding domain derived from human α7-nAChR and an ion pore domain derived from human GlyRα1. In some embodiments, the engineered receptor is CODA535 (SEQ ID NO: 60), and the control receptor is CODA71, CODA335, or CODA377.

**[0182]** In some embodiments, the engineered receptor comprises a ligand binding domain derived from human α7-nAChR and the amino acid substitutions corresponding to L131D and S172D in the LBD of human α7-nAChR. In some embodiments, such an engineered receptor has a lower potency (*e.g.*, as determined by a higher EC50) for acetylcholine as compared to a control receptor without such substitutions or with only one of such substitutions. In some embodiments, such an engineered receptor substantially retains the potency (or has a higher potency) for a non-native ligand as compared to a control receptor without such substitutions or with only one of such substitutions. In some embodiments, such

an engineered receptor has a higher potency (lower EC50) for CNL002 as compared to to a control receptor without such substitutions and/or with only one of such substitutions. In some embodiments, the non-native ligand for such an engineered receptor is CNL002, AZD-0328 or Facinicline. In some embodiments, the non-native ligand is Facinicline. In some embodiments, the non-native ligand is AZD-0328. In some embodiments, the non-native ligand is CNL002. In some embodiments, the engineered receptor is a chimeric LGIC comprising a ligand binding domain derived from human α7-nAChR and an ion pore domain derived from human GlyRα1. In some embodiments, the engineered receptor is CODA536 (SEQ ID NO: 58), and the control receptor is CODA71, CODA339, or CODA377.

**[0183]** In some embodiments, the engineered receptor comprises a ligand binding domain derived from human α7-nAChR and the amino acid substitutions corresponding to Y115D and S170T in the LBD of human α7-nAChR. In some embodiments, such an engineered receptor has a lower potency (*e.g.*, as determined by a higher EC50) for acetylcholine as compared to a control receptor without such substitutions and/or with only one of such substitutions (e.g., S170T only). In some embodiments, such an engineered receptor has an EC50 for acetylcholine of >3 mM. In some embodiments, such an engineered receptor substantially retains the potency (or has a higher potency) for a non-native ligand as compared to a control receptor without such substitutions or with only one of such substitutions (e.g., S170T only). In some embodiments, the non-native ligand for such an engineered receptor is Facinicline or TC-6987. In some embodiments, the non-native ligand is Facinicline. In some embodiments, the non-native ligand is TC-6987. In some embodiments, the engineered receptor is a chimeric LGIC comprising a ligand binding domain derived from human α7-nAChR and an ion pore domain derived from human GlyRα1. In some embodiments, the engineered receptor is CODA805 (SEQ ID NO: 63), and the control receptor is CODA71, CODA282, or CODA109.

**[0184]** In some embodiments, the engineered receptor comprises a ligand binding domain derived from human α7-nAChR and the amino acid substitutions corresponding to Y115D and L131Q in the LBD of human α7-nAChR. In some embodiments, such an engineered receptor has a lower potency (*e.g.*, as determined by a higher EC50) for acetylcholine as compared to a control receptor without such substitutions or with only one of such substitutions (e.g., L131Q only). In some embodiments, such an engineered receptor has an EC50 for acetylcholine of >3 mM. In some embodiments, such an engineered receptor substantially retains the potency (or has a higher potency) for a non-native ligand as compared to a control receptor without such substitutions or with only one of such substitutions. In some

embodiments, the non-native ligand for such an engineered receptor is AZD-0328, Facinicline, or TC-6987. In some embodiments, the non-native ligand is Facinicline. In some embodiments, the non-native ligand is AZD-0328. In some embodiments, the non-native ligand is TC-6987. In some embodiments, the engineered receptor is a chimeric LGIC comprising a ligand binding domain derived from human α7-nAChR and an ion pore domain derived from human GlyRα1. In some embodiments, the engineered receptor is CODA806 (SEQ ID NO: 62), and the control receptor is CODA71, CODA282, or CODA334.

**[0185]** In some embodiments, the engineered receptor comprises a ligand binding domain derived from human α7-nAChR and the amino acid substitutions corresponding to Y115D and L131E in the LBD of human α7-nAChR. In some embodiments, such an engineered receptor has a lower potency (*e.g.*, as determined by a higher EC50) for acetylcholine as compared to a control receptor without such substitutions or with only one of such substitutions. In some embodiments, such an engineered receptor substantially retains the potency (or has a higher potency) for a non-native ligand as compared to a control receptor without such substitutions or with only one of such substitutions. In some embodiments, the non-native ligand ligand for such an engineered receptor is TC-5619, AZD-0328, Facinicline or TC-6987. In some embodiments, the non-native ligand is TC-5619. In some embodiments, the non-native ligand is Facinicline. In some embodiments, the non-native ligand is AZD-0328. In some embodiments, the non-native ligand is TC-6987. In some embodiments, the engineered receptor is a chimeric LGIC comprising a ligand binding domain derived from human α7-nAChR and an ion pore domain derived from human GlyRα1. In some embodiments, the engineered receptor is CODA807 (SEQ ID NO: 61), and the control receptor is CODA71, CODA282, or CODA340.

**[0186]** In some embodiments, the engineered receptor comprises a ligand binding domain derived from human α7-nAChR and the amino acid substitutions corresponding to R101F and L131G in the LBD of human α7-nAChR. In some embodiments, such an engineered receptor has a lower potency (*e.g.*, as determined by a higher EC50) for acetylcholine as compared to a control receptor without such substitutions or with only one of such substitutions. In some embodiments, such an engineered receptor substantially retains the potency (or has a higher potency) for a non-native ligand as compared to a control receptor without such substitutions or with only one of such substitutions. In some embodiments, the non-native ligands for such an engineered receptor is CNL001, TC-5619, CNL002, AZD-0328, TC-6987, or Varenicline. In some embodiments, the non-native ligand is CNL001. In some embodiments, the non-native ligand is TC-5619. In some embodiments, the non-native ligand

is CNL002. In some embodiments, the non-native ligand is AZD-0328. In some embodiments, the non-native ligand is TC-6987. In some embodiments, the non-native ligand is Varenicline. In some embodiments, the engineered receptor is a chimeric LGIC comprising a ligand binding domain derived from human α7-nAChR and an ion pore domain derived from human GlyRα1. In some embodiments, the engineered receptor is CODA1025 (SEQ ID NO: 65), and the control receptor is CODA71, CODA236, or CODA325.

**[0187]** In some embodiments, the engineered receptor comprises a ligand binding domain derived from human α7-nAChR and the amino acid substitutions corresponding to R101F and L131D in the LBD of human α7-nAChR. In some embodiments, such an engineered receptor has a lower potency (*e.g.*, as determined by a higher EC50) for acetylcholine as compared to a control receptor without such substitutions or with only one of such substitutions. In some embodiments, such an engineered receptor substantially retains the potency (or has a higher potency) for a non-native ligand as compared to a control receptor without such substitutions or with only one of such substitutions. In some embodiments, the non-native ligands for such an engineered receptor is CNL001, TC-5619, CNL002, or TC-6987. In some embodiments, the non-native ligand is CNL001. In some embodiments, the non-native ligand is TC-5619. In some embodiments, the non-native ligand is CNL002. In some embodiments, the non-native ligand is TC-6987. In some embodiments, the engineered receptor is a chimeric LGIC comprising a ligand binding domain derived from human α7-nAChR and an ion pore domain derived from human GlyRα1. In some embodiments, the engineered receptor is CODA1027 (SEQ ID NO: 66), and the control receptor is CODA71, CODA236, or CODA339.

**[0188]** In some embodiments, the engineered receptor comprises a ligand binding domain derived from human α7-nAChR and the amino acid substitutions corresponding to Y115E and Y210W in the LBD of human α7-nAChR. In some embodiments, such an engineered receptor has a lower potency (*e.g.*, as determined by a higher EC50) for acetylcholine as compared to a control receptor without such substitutions or with only one of such substitutions. In some embodiments, such an engineered receptor substantially retains the potency (or has a higher potency) for a non-native ligand as compared to a control receptor without such substitutions or with only one of such substitutions. In some embodiments, the non-native ligand for such an engineered receptor is TC-5619, ABT-0126 or CNL002. In some embodiments, the non-native ligand is TC5619/Bradanicline. In some embodiments, the non-native ligand is ABT-0126. In some embodiments, the non-native ligand is CNL002. In some embodiments, the engineered receptor is a chimeric LGIC comprising a ligand binding domain

derived from human α7-nAChR and an ion pore domain derived from human GlyRα1. In some embodiments, the engineered receptor is CODA1039 (SEQ ID NO: 67), and the control receptor is CODA71, CODA283, or CODA409.

**[0189]** In some embodiments, the engineered receptor comprises a ligand binding domain derived from human α7-nAChR and the amino acid substitutions corresponding to R101W and Y210V in the LBD of human α7-nAChR. In some embodiments, such an engineered receptor has a lower potency (*e.g.*, as determined by a higher EC50) for acetylcholine as compared to a control receptor without such substitutions or with only one of such substitutions. In some embodiments, such an engineered receptor has an EC50 for acetylcholine of >3 mM. In some embodiments, such an engineered receptor substantially retains the potency (or has a higher potency) for a non-native ligand as compared to a control receptor without such substitutions or with only one of such substitutions. In some embodiments, the non-native ligand is TC-5619/bradanicline. In some embodiments, the engineered receptor is a chimeric LGIC comprising a ligand binding domain derived from human α7-nAChR and an ion pore domain derived from human GlyRα1. In some embodiments, the engineered receptor is CODA1045 (SEQ ID NO: 68), and the control receptor is CODA71, CODA238, or CODA405.

**[0190]** In some embodiments, the engineered receptor comprises a ligand binding domain derived from human α7-nAChR and the amino acid substitutions corresponding to R101F and Y210V in the LBD of human α7-nAChR. In some embodiments, such an engineered receptor has a lower potency (*e.g.*, as determined by a higher EC50) for acetylcholine as compared to a control receptor without such substitutions or with only one of such substitutions (e.g., R101F). In some embodiments, such an engineered receptor has an EC50 for acetylcholine of >3 mM. In some embodiments, such an engineered receptor substantially retains the potency (or has a higher potency) for a non-native ligand as compared to a control receptor without such substitutions or with only one of such substitutions (e.g., Y210V). In some embodiments, the non-native ligand is TC-5619/Bradanicline. In some embodiments, the engineered receptor is a chimeric LGIC comprising a ligand binding domain derived from human α7-nAChR and an ion pore domain derived from human GlyRα1. In some embodiments, the engineered receptor is CODA1047 (SEQ ID NO: 69), and the control receptor is CODA71, CODA236, or CODA405.

**[0191]** In some embodiments, the engineered receptor comprises a ligand binding domain derived from human α7-nAChR and the amino acid substitutions corresponding to R101F and Y210F in the LBD of human α7-nAChR. In some embodiments, such an engineered

receptor has a lower potency (*e.g.*, as determined by a higher EC50) for acetylcholine as compared to a control receptor without such substitutions or with only one of such substitutions. In some embodiments, such an engineered receptor has an EC50 for acetylcholine of >1 mM. In some embodiments, such an engineered receptor substantially retains the potency (or has a higher potency) for a non-native ligand as compared to a control receptor without such substitutions or with only one of such substitutions. In some embodiments, such an engineered receptor has an EC50 of <= about 10 nM for CNL001 and/or an EC50 of <= about 30 nM for TC5619/Bradanicline. In some embodiments, the non-native ligand for such an engineered receptor is CNL001 or TC5619/Bradanicline. In some embodiments, the non-native ligand is CNL001. In some embodiments, the non-native ligand is TC5619/Bradanicline. In some embodiments, the engineered receptor is a chimeric LGIC comprising a ligand binding domain derived from human α7-nAChR and an ion pore domain derived from human GlyRα1. In some embodiments, the engineered receptor is CODA1048 (SEQ ID NO: 70), and the control receptor is CODA71, CODA236, or CODA407.

**[0192]** In some embodiments, the engineered receptor comprises a ligand binding domain derived from human α7-nAChR and the amino acid substitutions corresponding to R101M and L131A in the LBD of human α7-nAChR. In some embodiments, such an engineered receptor has a lower potency (*e.g.*, as determined by a higher EC50) for acetylcholine as compared to a control receptor without such substitutions or with only one of such substitutions. In some embodiments, such an engineered receptor has an EC50 for acetylcholine of >1 mM. In some embodiments, such an engineered receptor substantially retains the potency (or has a higher potency) for a non-native ligand as compared to a control receptor without such substitutions or with only one of such substitutions. In some embodiments, such an engineered receptor has an EC50 of <= about 10 nM for CNL001, an EC50 of <= about 3 nM for TC5619/Bradanicline, and/or an EC50 of <= about 3 nM for Varenicline. In some embodiments, the non-native ligand for such an engineered receptor is CNL001, TC5619/Bradanicline, or Varenicline. In some embodiments, the non-native ligand is CNL001. In some embodiments, the non-native ligand is TC5619/Bradanicline. In some embodiments, the non-native ligand is Varenicline. In some embodiments, the engineered receptor is a chimeric LGIC comprising a ligand binding domain derived from human α7-nAChR and an ion pore domain derived from human GlyRα1. In some embodiments, the engineered receptor is CODA1053 (SEQ ID NO: 71), and the control receptor is CODA71, CODA237, or CODA326.

**[0193]** In some embodiments, the engineered receptor comprises a ligand binding domain derived from human α7-nAChR and the amino acid substitutions corresponding to R101M and L131F in the LBD of human α7-nAChR. In some embodiments, such an engineered receptor has a lower potency (*e.g.*, as determined by a higher EC50) for acetylcholine as compared to a control receptor without such substitutions or with only one of such substitutions. In some embodiments, such an engineered receptor has an EC50 for acetylcholine of >1 mM or >3 mM. In some embodiments, such an engineered receptor substantially retains the potency (or has a higher potency) for a non-native ligand as compared to a control receptor without such substitutions or with only one of such substitutions In some embodiments, such an engineered receptor has an EC50 of <= about 1 nM for CNL001, and/or an EC50 of <= about 3 nM for Varenicline. In some embodiments, the non-native ligand is CNL001, TC5619/Bradanicline, or Varenicline. In some embodiments, the non-native ligand is CNL001. In some embodiments, the non-native ligand is TC5619/Bradanicline. In some embodiments, the non-native ligand is Varenicline. In some embodiments, the engineered receptor is a chimeric LGIC comprising a ligand binding domain derived from human α7-nAChR and an ion pore domain derived from human GlyRα1. In some embodiments, the engineered receptor is CODA1054 (SEQ ID NO: 72), and the control receptor is CODA71, CODA237, or CODA330.

**[0194]** In some embodiments, the engineered receptor comprises a ligand binding domain derived from human α7-nAChR and the amino acid substitutions corresponding to R101W, Y115E and Y210W in the LBD of human α7-nAChR. In some embodiments, such an engineered receptor has a lower potency (*e.g.*, as determined by a higher EC50) for acetylcholine as compared to a control receptor without such substitutions or with only one or two of such substitutions. In some embodiments, such an engineered receptor has an EC50 for acetylcholine of >3 mM or >10 mM. In some embodiments, such an engineered receptor substantially retains the potency (or has a higher potency) for a non-native ligand as compared to a control receptor without such substitutions or with only one or two of such substitutions. In some embodiments, the non-native ligand is TC-5619/Bradanicline. In some embodiments, such an engineered receptor has an EC50 of <= about 1 nM for TC-5619/Bradanicline. In some embodiments, the engineered receptor is a chimeric LGIC comprising a ligand binding domain derived from human α7-nAChR and an ion pore domain derived from human GlyRα1. In some embodiments, the engineered receptor is CODA1055 (SEQ ID NO: 73), and the control receptor is CODA71, CODA238, CODA283, or CODA409.

**[0195]** In some embodiments, the engineered receptor comprises a ligand binding domain derived from human α7-nAChR and the amino acid substitutions corresponding to R101F, Y115E and Y210W in the LBD of human α7-nAChR. In some embodiments, such an engineered receptor has a lower potency (*e.g.*, as determined by a higher EC50) for acetylcholine as compared to a control receptor without such substitutions or with only one or two of such substitutions. In some embodiments, such an engineered receptor has an EC50 for acetylcholine of >3 mM or >10 mM. In some embodiments, such an engineered receptor substantially retains the potency (or has a higher potency) for a non-native ligand as compared to a control receptor without such substitutions or with only one or two of such substitutions. In some embodiments, the non-native ligand is TC-5619/Bradanicline. In some embodiments, such an engineered receptor has an EC50 of <= about 10 nM for TC-5619/Bradanicline. In some embodiments, the engineered receptor is a chimeric LGIC comprising a ligand binding domain derived from human α7-nAChR and an ion pore domain derived from human GlyRα1. In some embodiments, the engineered receptor is CODA1056 (SEQ ID NO: 74), and the control receptor is CODA71, CODA236, CODA283, or CODA409.

**[0196]** In some embodiments, the engineered receptor comprises a ligand binding domain derived from human α7-nAChR and the amino acid substitutions corresponding to W77F, R101F and L131D in the LBD of human α7-nAChR. In some embodiments, such an engineered receptor has a lower potency (*e.g.*, as determined by a higher EC50) for acetylcholine as compared to a control receptor without such substitutions or with only one or two of such substitutions. In some embodiments, such an engineered receptor has an EC50 for acetylcholine of >1 mM or >3 mM. In some embodiments, such an engineered receptor substantially retains the potency (or has a higher potency) for a non-native ligand as compared to a control receptor without such substitutions or with only one or two of such substitutions. In some embodiments, such an engineered receptor has an EC50 of <= about 10 nM for CNL002. In some embodiments, the non-native ligand is CNL001, CNL002, or ABT-126. In some embodiments, the non-native ligand is CNL001. In some embodiments, the non-native ligand is CNL002. In some embodiments, the non-native ligand is ABT-126. In some embodiments, the engineered receptor is a chimeric LGIC comprising a ligand binding domain derived from human α7-nAChR and an ion pore domain derived from human GlyRα1. In some embodiments, the engineered receptor is CODA1138 (SEQ ID NO: 75), and the control receptor is CODA71, CODA217, CODA236, or CODA339.

**[0197]** In some embodiments, the engineered receptor comprises a ligand binding domain derived from human α7-nAChR and the amino acid substitutions corresponding to

R101F, L131N, and S172D in the LBD of human α7-nAChR. In some embodiments, such an engineered receptor has a lower potency (*e.g.*, as determined by a higher EC50) for acetylcholine as compared to a control receptor without such substitutions or with only one or two of such substitutions. In some embodiments, such an engineered receptor has an EC50 for acetylcholine of >1 mM or >3 mM. In some embodiments, such an engineered receptor substantially retains the potency (or has a higher potency) for a non-native ligand as compared to a control receptor without such substitutions or with only one or two of such substitutions. In some embodiments, such an engineered receptor has an EC50 of <= about 1 nM for CNL001, or an EC50 of <= about 10 nM for CNL002. In some embodiments, the non-native ligand is CNL001 or CNL002. In some embodiments, the non-native ligand is CNL001. In some embodiments, the non-native ligand is CNL002. In some embodiments, the engineered receptor is a chimeric LGIC comprising a ligand binding domain derived from human α7-nAChR and an ion pore domain derived from human GlyRα1. In some embodiments, the engineered receptor is CODA1140 (SEQ ID NO: 76), and the control receptor is CODA71, CODA236, CODA337, or CODA377.

**[0198]** In some embodiments, the engineered receptor comprises a ligand binding domain derived from human α7-nAChR and the amino acid substitutions corresponding to Q139E and S172D in the LBD of human α7-nAChR. In some embodiments, such an engineered receptor has a lower potency (*e.g.*, as determined by a higher EC50) for acetylcholine as compared to a control receptor without such substitutions or with only one of such substitutions (e.g., S172D). In some embodiments, such an engineered receptor substantially retains the potency (or has a higher potency) for a non-native ligand as compared to a control receptor without such substitutions or with only one of such substitutions. In some embodiments, the non-native ligand is CNL001 or CNL002. In some embodiments, the non-native ligand is CNL001. In some embodiments, the non-native ligand is CNL002. In some embodiments, the engineered receptor is a chimeric LGIC comprising a ligand binding domain derived from human α7-nAChR and an ion pore domain derived from human GlyRα1. In some embodiments, the engineered receptor is CODA1157 (SEQ ID NO: 77), and the control receptor is CODA71, CODA945, or CODA377.

**[0199]** In some embodiments, the engineered receptor comprises a ligand binding domain derived from human α7-nAChR and the amino acid substitutions corresponding to S172D and Y210W in the LBD of human α7-nAChR. In some embodiments, such an engineered receptor has a lower potency (*e.g.*, as determined by a higher EC50) for acetylcholine as compared to a control receptor without such substitutions or with only one of

such substitutions. In some embodiments, such an engineered receptor has an EC50 for acetylcholine of >3 mM or >10 mM. In some embodiments, such an engineered receptor substantially retains the potency (or hasa higher potency) for a non-native ligand as compared to a control receptor without such substitutions or with only one of such substitutions. In some embodiments, such an engineered receptor has an EC50 of <= about 10 nM for CNL001. In some embodiments, the non-native ligand for such an engineered receptor is CNL001, CNL002, or ABT-126. In some embodiments, the non-native ligand is CNL001. In some embodiments, the non-native ligand is CNL002. In some embodiments, the non-native ligand is ABT-126. In some embodiments, the engineered receptor is a chimeric LGIC comprising a ligand binding domain derived from human α7-nAChR and an ion pore domain derived from human GlyRα1. In some embodiments, the engineered receptor is CODA1173 (SEQ ID NO: 78), and the control receptor is CODA71, CODA377, or CODA409.

**[0200]** In some embodiments, the engineered receptor comprises a ligand binding domain derived from human α7-nAChR and an amino acid substitution at the amino acid residue corresponding to Y140 of human α7-nAChR. In some embodiments, the amino acid substitution is Y140I. In some embodiments, such an engineered receptor has a lower potency (*e.g.*, as determined by a higher EC50) for acetylcholine as compared to a control receptor without such a substitution. In some embodiments, the engineered receptor is a chimeric LGIC comprising a ligand binding domain derived from human α7-nAChR and an ion pore domain derived from human GlyRα1 (e.g., CODA952, SEQ ID NO: 64) and the control receptor is CODA71 (SEQ ID NO: 33). In some embodiments, such an engineered receptor substantially retains the potency (or has a higher potency) for a non-native ligand as compared to a control receptor without such a substitution. In some embodiments, the non-native ligand for such an engineered receptor is CNL001, TC-5619/Bradanicline, CNL002, or Facinicline. In some embodiments, the non-native ligand is CNL001. In some embodiments, the non-native ligand is TC-5619/Bradanicline. In some embodiments, the non-native ligand is CNL002. In some embodiments, the non-native ligand is Facinicline.

**[0201]** In some embodiments, the engineered receptor comprises a ligand binding domain derived from human α7-nAChR and an amino acid substitution at the amino acid residue corresponding to Y140 of human α7-nAChR. In some embodiments, the amino acid substitution is Y140C. In some embodiments, such an engineered receptor has a lower potency (*e.g.*, as determined by a higher EC50) for acetylcholine as compared to a control receptor without such a substitution. In some embodiments, the engineered receptor is a chimeric LGIC comprising a ligand binding domain derived from human α7-nAChR and an ion pore domain

derived from human GlyRα1 (e.g., CODA965, SEQ ID NO: 88) and the control receptor is CODA71 (SEQ ID NO: 33). In some embodiments, such an engineered receptor substantially retains the potency (or has a higher potency) for a non-native ligand as compared to a control receptor without such a substitution. In some embodiments, the non-native ligand for such an engineered receptor is CNL001, TC-5619/Bradanicline, CNL002, ABT-126, or TC-6987. In some embodiments, the non-native ligand is CNL001. In some embodiments, the non-native ligand is TC-5619/Bradanicline. In some embodiments, the non-native ligand is CNL002. In some embodiments, the non-native ligand is ABT-126. In some embodiments, the non-native ligand is TC-6987.

**[0202]** In some embodiments, the engineered receptor comprises a ligand binding domain derived from human α7-nAChR and an amino acid substitution at the amino acid residue corresponding to Y140 of human α7-nAChR. In some embodiments, the engineered receptor comprises a ligand binding domain derived from human α7-nAChR and the amino acid substitutions corresponding to Y140R in the LBD of human α7-nAChR. In some embodiments, such an engineered receptor has a lower potency (*e.g.*, as determined by a higher EC50) for acetylcholine as compared to a control receptor without such substitutions or with only one or two of such substitutions. In some embodiments, such an engineered receptor has an EC50 for acetylcholine of >0.4 mM, >1 mM or >3 mM. In some embodiments, such an engineered receptor substantially retains the potency (or has a higher potency) for a non-native ligand as compared to a control receptor without such substitutions or with only one or two of such substitutions. In some embodiments, the non-native ligand is TC-5619. In some embodiments, the engineered receptor is a chimeric LGIC comprising a ligand binding domain derived from human α7-nAChR and an ion pore domain derived from human GlyRα1. In some embodiments, the engineered receptor is CODA967 (SEQ ID NO: 89), and the control receptor is CODA71.

**[0203]** In some embodiments, the engineered receptor comprises a ligand binding domain derived from human α7-nAChR and the amino acid substitutions corresponding to Y115E and L131M in the LBD of human α7-nAChR. In some embodiments, such an engineered receptor has a lower potency (*e.g.*, as determined by a higher EC50) for acetylcholine as compared to a control receptor without such substitutions or with only one or two of such substitutions. In some embodiments, such an engineered receptor has an EC50 for acetylcholine of >0.4 mM, >1 mM or >3 mM. In some embodiments, such an engineered receptor substantially retains the potency (or has a higher potency) for a non-native ligand as compared to a control receptor without such substitutions or with only one or two of such

substitutions. In some embodiments, the non-native ligand is ABT-126. In some embodiments, the engineered receptor is a chimeric LGIC comprising a ligand binding domain derived from human α7-nAChR and an ion pore domain derived from human GlyRα1. In some embodiments, the engineered receptor is CODA1101 (SEQ ID NO: 90), and the control receptor is CODA71.

**[0204]** In some embodiments, the engineered receptor comprises a ligand binding domain derived from human α7-nAChR and the amino acid substitutions corresponding to Q139W and S172D in the LBD of human α7-nAChR. In some embodiments, such an engineered receptor has a lower potency (*e.g.*, as determined by a higher EC50) for acetylcholine as compared to a control receptor without such substitutions or with only one or two of such substitutions. In some embodiments, such an engineered receptor has an EC50 for acetylcholine of >0.4 mM, >1 mM or >3 mM. In some embodiments, such an engineered receptor substantially retains the potency (or has a higher potency) for a non-native ligand as compared to a control receptor without such substitutions or with only one or two of such substitutions. In some embodiments, the non-native ligand is ABT-126 or CNL002. In some embodiments, the non-native ligand is ABT-126. In some embodiments, the non-native ligand is CNL002. In some embodiments, the engineered receptor is a chimeric LGIC comprising a ligand binding domain derived from human α7-nAChR and an ion pore domain derived from human GlyRα1. In some embodiments, the engineered receptor is CODA1167 (SEQ ID NO: 91), and the control receptor is CODA71.

**[0205]** In some embodiments, the engineered receptor comprises a ligand binding domain derived from human α7-nAChR and the amino acid substitutions corresponding to Q139W and Y210W in the LBD of human α7-nAChR. In some embodiments, such an engineered receptor has a lower potency (*e.g.*, as determined by a higher EC50) for acetylcholine as compared to a control receptor without such substitutions or with only one or two of such substitutions. In some embodiments, such an engineered receptor has an EC50 for acetylcholine of >0.4 mM, >1 mM or >3 mM. In some embodiments, such an engineered receptor substantially retains the potency (or has a higher potency) for a non-native ligand as compared to a control receptor without such substitutions or with only one or two of such substitutions. In some embodiments, the non-native ligand is ABT-126. In some embodiments, the engineered receptor is a chimeric LGIC comprising a ligand binding domain derived from human α7-nAChR and an ion pore domain derived from human GlyRα1. In some embodiments, the engineered receptor is CODA1168 (SEQ ID NO: 92), and the control receptor is CODA71.

**[0206]** In some embodiments, the engineered receptor comprises a ligand binding domain derived from human α7-nAChR and the amino acid substitutions corresponding to Q139W, S172D, and Y217I in the LBD of human α7-nAChR. In some embodiments, such an engineered receptor has a lower potency (*e.g.*, as determined by a higher EC50) for acetylcholine as compared to a control receptor without such substitutions or with only one or two of such substitutions. In some embodiments, such an engineered receptor has an EC50 for acetylcholine of >0.4 mM, >1 mM or >3 mM. In some embodiments, such an engineered receptor substantially retains the potency (or has a higher potency) for a non-native ligand as compared to a control receptor without such substitutions or with only one or two of such substitutions. In some embodiments, the non-native ligand is ABT-126 or CNL001. In some embodiments, the non-native ligand is ABT-126. In some embodiments, the non-native ligand is CNL001. In some embodiments, the engineered receptor is a chimeric LGIC comprising a ligand binding domain derived from human α7-nAChR and an ion pore domain derived from human GlyRα1. In some embodiments, the engineered receptor is CODA1245 (SEQ ID NO: 93, and the control receptor is CODA71.

**[0207]** In some embodiments, the engineered receptor comprises a ligand binding domain derived from human α7-nAChR and the amino acid substitutions corresponding to Y115E, Q139E, and S172D in the LBD of human α7-nAChR. In some embodiments, such an engineered receptor has a lower potency (*e.g.*, as determined by a higher EC50) for acetylcholine as compared to a control receptor without such substitutions or with only one or two of such substitutions. In some embodiments, such an engineered receptor has an EC50 for acetylcholine of >0.4 mM, >1 mM or >3 mM. In some embodiments, such an engineered receptor substantially retains the potency (or has a higher potency) for a non-native ligand as compared to a control receptor without such substitutions or with only one or two of such substitutions. In some embodiments, the non-native ligand is CNL001. In some embodiments, the engineered receptor is a chimeric LGIC comprising a ligand binding domain derived from human α7-nAChR and an ion pore domain derived from human GlyRα1. In some embodiments, the engineered receptor is CODA1261 (SEQ ID NO: 94), and the control receptor is CODA71.

**[0208]** In some embodiments, the engineered receptor comprises a ligand binding domain derived from human α7-nAChR and the amino acid substitutions corresponding to R101F, L131G, Q139L, and Y217F in the LBD of human α7-nAChR. In some embodiments, such an engineered receptor has a lower potency (*e.g.*, as determined by a higher EC50) for acetylcholine as compared to a control receptor without such substitutions or with only one or

two of such substitutions. In some embodiments, such an engineered receptor has an EC50 for acetylcholine of >0.4 mM, >1 mM or >3 mM. In some embodiments, such an engineered receptor substantially retains the potency (or has a higher potency) for a non-native ligand as compared to a control receptor without such substitutions or with only one or two of such substitutions. In some embodiments, the non-native ligand is Varenicline. In some embodiments, the engineered receptor is a chimeric LGIC comprising a ligand binding domain derived from human α7-nAChR and an ion pore domain derived from human GlyRα1. In some embodiments, the engineered receptor is CODA1458 (SEQ ID NO: 95), and the control receptor is CODA71.

**[0209]** In some embodiments, the engineered receptor comprises a ligand binding domain derived from human α7-nAChR and the amino acid substitutions corresponding to Y115Q, and S172D in the LBD of human α7-nAChR. In some embodiments, such an engineered receptor has a lower potency (*e.g.*, as determined by a higher EC50) for acetylcholine as compared to a control receptor without such substitutions or with only one or two of such substitutions. In some embodiments, such an engineered receptor has an EC50 for acetylcholine of >0.4 mM, >1 mM or >3 mM. In some embodiments, such an engineered receptor substantially retains the potency (or has a higher potency) for a non-native ligand as compared to a control receptor without such substitutions or with only one or two of such substitutions. In some embodiments, the non-native ligand is CNL001. In some embodiments, the engineered receptor is a chimeric LGIC comprising a ligand binding domain derived from human α7-nAChR and an ion pore domain derived from human GlyRα1. In some embodiments, the engineered receptor is CODA1471 (SEQ ID NO: 96), and the control receptor is CODA71.

**[0210]** In some embodiments, the engineered receptor comprises a ligand binding domain derived from human α7-nAChR and the amino acid substitutions corresponding to L141M, S172D, and Y210W in the LBD of human α7-nAChR. In some embodiments, such an engineered receptor has a lower potency (*e.g.*, as determined by a higher EC50) for acetylcholine as compared to a control receptor without such substitutions or with only one or two of such substitutions. In some embodiments, such an engineered receptor has an EC50 for acetylcholine of >0.4 mM, >1 mM or >3 mM. In some embodiments, such an engineered receptor substantially retains the potency (or has a higher potency) for a non-native ligand as compared to a control receptor without such substitutions or with only one or two of such substitutions. In some embodiments, the non-native ligand is CNL001 or CNL002. In some embodiments, the non-native ligand is CNL001. In some embodiments, the non-native ligand

is CNL002. In some embodiments, the engineered receptor is a chimeric LGIC comprising a ligand binding domain derived from human α7-nAChR and an ion pore domain derived from human GlyRα1. In some embodiments, the engineered receptor is CODA1500 (SEQ ID NO: 97), and the control receptor is CODA71.

**[0211]** In some embodiments, the engineered receptor comprises a ligand binding domain derived from human α7-nAChR and the amino acid substitutions corresponding to R101F, L131N, and Y217F in the LBD of human α7-nAChR. In some embodiments, such an engineered receptor has a lower potency (*e.g.*, as determined by a higher EC50) for acetylcholine as compared to a control receptor without such substitutions or with only one or two of such substitutions. In some embodiments, such an engineered receptor has an EC50 for acetylcholine of >0.4 mM, >1 mM or >3 mM. In some embodiments, such an engineered receptor substantially retains the potency (or has a higher potency) for a non-native ligand as compared to a control receptor without such substitutions or with only one or two of such substitutions. In some embodiments, the non-native ligand is Varenicline or CNL002. In some embodiments, the non-native ligand is Varenicline. In some embodiments, the non-native ligand is CNL002. In some embodiments, the engineered receptor is a chimeric LGIC comprising a ligand binding domain derived from human α7-nAChR and an ion pore domain derived from human GlyRα1. In some embodiments, the engineered receptor is CODA1507 (SEQ ID NO: 98), and the control receptor is CODA71.

**[0212]** A summary of these exemplary engineered receptors is provided below in Table 10.

**Table 10: Exemplary Engineered Receptors**

| SEQ ID NO: | Name | Sequence |
|---|---|---|
| SEQ ID NO: 58 | CODA536 | L131D, S172D in SEQ ID NO: 33 |
| SEQ ID NO: 59 | CODA534 | L131S, S172D in SEQ ID NO: 33 |
| SEQ ID NO: 60 | CODA535 | L131T, S172D in SEQ ID NO: 33 |
| SEQ ID NO: 61 | CODA807 | Y115D, L131E in SEQ ID NO: 33 |
| SEQ ID NO: 62 | CODA806 | Y115D, L131Q in SEQ ID NO: 33 |
| SEQ ID NO: 63 | CODA805 | Y115D, S170T in SEQ ID NO: 33 |
| SEQ ID NO: 64 | CODA952 | Y140I in SEQ ID NO: 33 |
| SEQ ID NO: 65 | CODA1025 | R101F, L131G in SEQ ID NO: 33 |
| SEQ ID NO: 66 | CODA1027 | R101F, L131D in SEQ ID NO: 33 |

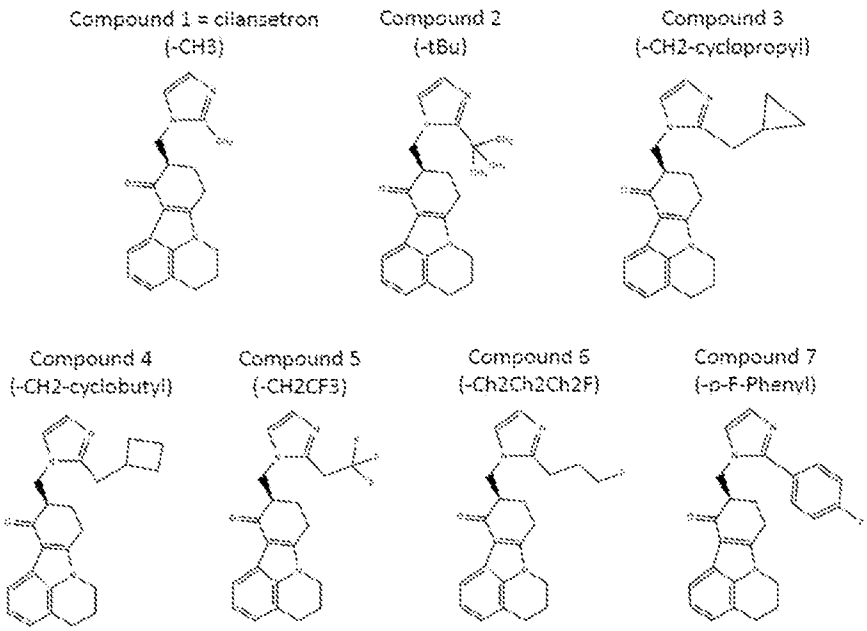
| SEQ ID NO: | Name | Sequence |
|---|---|---|
| SEQ ID NO: 67 | CODA1039 | Y115E, Y210W in SEQ ID NO: 33 |
| SEQ ID NO: 68 | CODA1045 | R101W, Y210V in SEQ ID NO: 33 |
| SEQ ID NO: 69 | CODA1047 | R101F, Y210V in SEQ ID NO: 33 |
| SEQ ID NO: 70 | CODA1048 | R101F, Y210F in SEQ ID NO: 33 |
| SEQ ID NO: 71 | CODA1053 | R101M, L131A in SEQ ID NO: 33 |
| SEQ ID NO: 72 | CODA1054 | R101M, L131F in SEQ ID NO: 33 |
| SEQ ID NO: 73 | CODA1055 | R101W, Y115E, Y210W in SEQ ID NO: 33 |
| SEQ ID NO: 74 | CODA1056 | R101F, Y115E, Y210W in SEQ ID NO: 33 |
| SEQ ID NO: 75 | CODA1138 | W77F, R101F, L131D in SEQ ID NO: 33 |
| SEQ ID NO: 76 | CODA1140 | R101F, L131N, S172D in SEQ ID NO: 33 |
| SEQ ID NO: 77 | CODA1157 | Q139E, S172D in SEQ ID NO: 33 |
| SEQ ID NO: 78 | CODA1173 | S172D, Y210W in SEQ ID NO: 33 |
| SEQ ID NO: 88 | CODA965 | Y140C in SEQ ID NO: 33 |
| SEQ ID NO: 89 | CODA967 | Y140R in SEQ ID NO: 33 |
| SEQ ID NO: 90 | CODA1101 | Y115E, L131M in SEQ ID NO: 33 |
| SEQ ID NO: 91 | CODA1167 | Q139W, S172D in SEQ ID NO: 33 |
| SEQ ID NO: 92 | CODA1168 | Q139W, Y210W in SEQ ID NO: 33 |
| SEQ ID NO: 93 | CODA1245 | Q139W, S172D, Y217I in SEQ ID NO: 33 |
| SEQ ID NO: 94 | CODA1261 | Y115E, Q139E, S172D in SEQ ID NO: 33 |
| SEQ ID NO: 95 | CODA1458 | R101F, L131G, Q139L, Y217F in SEQ ID NO: 33 |
| SEQ ID NO: 96 | CODA1471 | Y115Q, S172D in SEQ ID NO: 33 |
| SEQ ID NO: 97 | CODA1500 | L141M, S172D, Y210W in SEQ ID NO: 33 |
| SEQ ID NO: 98 | CODA1507 | R101F, L131N, Y217F in SEQ ID NO: 33 |

**D. Ligands**

**[0213]** In some embodiments, the ligands of the disclosure refer to exogenous drugs or compounds with a known mechanism of action on a mammalian cell (*e.g.*, are known to act as an agonist, antagonist, or modulator of a receptor). Such ligands may also be referred to as "binding agents". Ligands of the disclosure can include proteins, lipids, nucleic acids, and/or small molecules. In some embodiments, ligands include drugs or compounds that have been

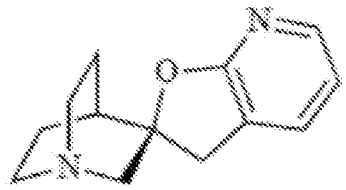
approved by the US Food and Drug Administration (FDA) for clinical use in the treatment of a particular disease (*e.g.*, a neurological disease). In some embodiments, ligands include drugs or compounds that have not been approved by the FDA for clinical use, but have been tested in one or more clinical trials, are currently being tested in one or more clinical trials, and/or are anticipated to be tested in one or more clinical trials. In some embodiments, ligands include drugs or compounds that have not been approved by the FDA for clinical use, but are routinely used in laboratory research. In some embodiments, the ligand is an analog of one of the aforementioned ligands. In particular embodiments, a ligand is selected from any one of the ligands in **Tables 2 – 9** below. In some embodiments, the ligand is selected from the group consisting of AZD0328, ABT-126, AQW-051, Cannabidiol, Cilansetron, PH-399733, FACINICLINE/RG3487/MEM-3454, TC-6987, CNL002, and TC-5619/AT-101. In some embodiments, the ligand is selected from the group consisting of ABT-126, AZD-0328, CNL002, RG3487, TC-6987, CNL001, TC-6683, Varenicline, and TC-5619.

**[0214]** In particular embodiments, the ligand is an analog of Cilansetron, *e.g.* as described by one of the compound formulas 2-7 below in either its R or S enantiomer:



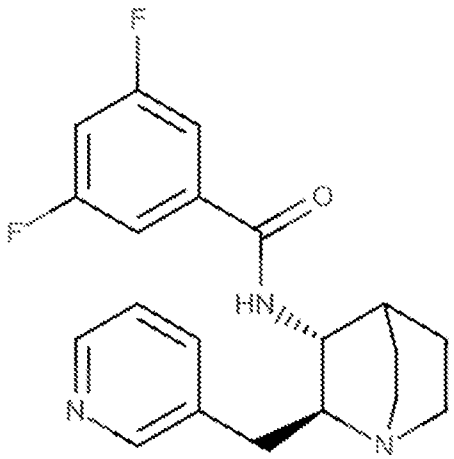

**[0215]** In some embodiments, the ligand acts as an agonist. The term "agonist" as used herein refers to a ligand that induces a signaling response. In some embodiments, the ligand acts as an antagonist. The term antagonist is used herein to refer to a ligand that inhibits a signaling response.

**[0216]** In some embodiments, the ligand is AZD-0328 according to the formula below:

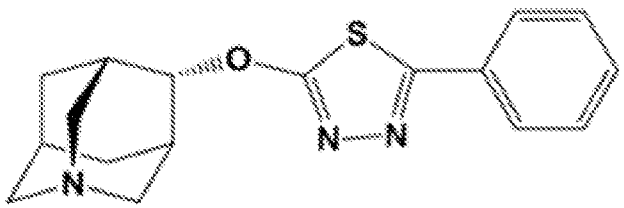


AZD0328

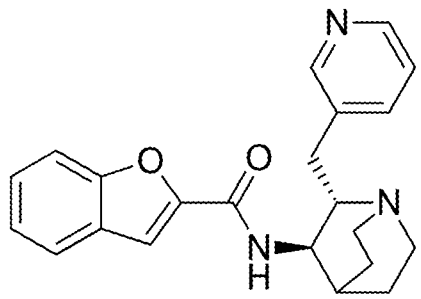
**[0217]** In some embodiments, the ligand is TC-6987 according to the formula below:



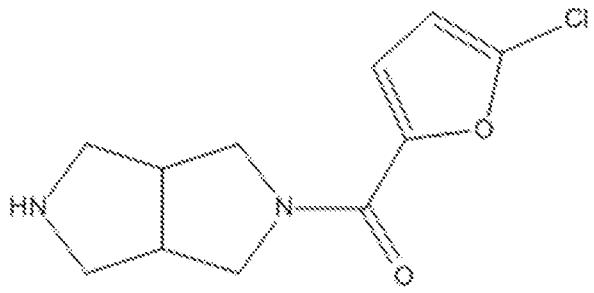
**[0218]** In some embodiments, the ligand is ABT-126 according to the formula below:



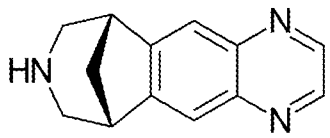
**[0219]** In some embodiments, the ligand is TC-5619/Bradanicline according to the formula below:



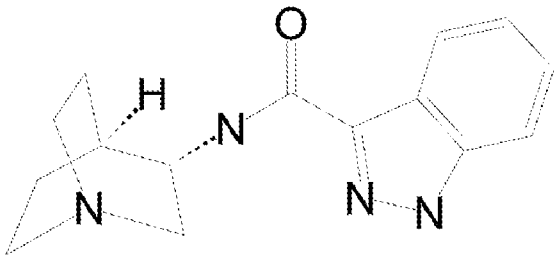
**[0220]** In some embodiments, the ligand is TC-6683 according to the formula below:




**[0221]** In some embodiments, the ligand is Varenicline according to the formula below:




**[0222]** In some embodiments, the ligand is Facinicline/RG3487 according to the formula below:




**[0223]** In some embodiments, the ligand is CNL001.

**[0224]** In some embodiments, the ligand is CNL002.

**[0225]** In some embodiments, the ligand is an anxiolytic, anticonvulsant, antidepressant, antipsychotic, antiemetic, nootropic, antibiotic, antifungal, antiviral, or an antiparasitic.

**Table 2: Ligands for Glycine Receptor (GlyR)**

| Agonists | | Modulators/Binders | Antagonists | |
|---|---|---|---|---|
| Bilobalide | L-Serine | Gavestinel | 468816 | Lindane |
| Cannabidiol | MDL-27531 | Halothane | ACEA-2085 | MDL-100748 |
| D-Alanine | Methoxyflurane | | Bicuculline | MDL-102288 |
| D-Serine | Milacemide | | Brucine | MDL-105519 |
| Desflurane | Moxidectin | | Caffeine | PD-165650 |
| Doramectin | NRX-1050 | | Gavestinel | Picrotoxin |
| Emamectin | NRX-1060 | | Ginkgo biloba | Strychnine |
| Eprinomectin | P-9939 | | GV-196771 | Thiocolchicoside |
| Ethanol | Quisqualamine | | GW 468816 | Tutin |

| | | | | |
|---|---|---|---|---|
| Glycine | Rapastinel | | HMR-2371 | UK-315716 |
| Hypotaurine | S-18841 | | L-695902 | ZD-9379 |
| Isoflurane | Sarcosine | | L-701324 | |
| Ivermectin | Sevoflurane | | | |
| L-Alanine | Taurine | | | |
| L-Proline | β-Alanine | | | |

**Table 3: Ligands for λ-Aminobutyric Acid A Receptor (GABA-A)**

| Agonists | Modulators/Binders | | | Antagonists |
|---|---|---|---|---|
| 3-acyl-4-quinolone | (−)Epigallo-catechin-3-Gallate | Etifoxine | Pentobarbital | (±)-cis-(3-Aminocyclopentyl) butylphosphinic acid |
| Acamprosate | 10-Methoxy-yangonin | Etizolam | Petrichloral | (S)-(4-Aminocyclopent-1-enyl)butylphosphinic acid |
| Alfadolone | 11-Hydroxy-yangonin | Etomidate | PF-4480682 | Amoxapine |
| Bamaluzole | 11-Methoxy-12-Hydroxy-dehydrokavain | Evt-201 | Phenazepam | Bicuculline |
| Basmisanil | 11-Methoxy-yangonin | Fasiplon | Phenobarbital | CGP-36742 (3-aminopropyl-n-butyl-phosphinic acid) |
| Bretazenil | 123i-Iomazenil | Fg-8205 | Pinazepam | Flumazenil |
| CACA | 2-Oxoquazepam | Fletazepam | Pipequaline | Gabazine |
| CAMP | 3-Hydroxy-phenazepam | Flubromazepam | Pivoxazepam | Ginkgo biloba |
| CP-409092 | 5-Hydroxykavain | Flubromazolam | Potassium Bromide | Lindane |
| Doramectin | 5,6-Dehydro-methysticin | Fludiazepam | Prazepam | Methohexital |
| Emamectin | 5,6-Dihydro-yangonin | Flumazenil | Premazepam | Picrotoxin |
| Eprinomectin | 5,6,7,8-Tetrahydro-yangonin | Flunitrazepam | Primidone | SKF-97541 (3-Aminopropyl (methyl)phosphinic acid) |
| Eszopiclone | 7,8-Dihydrokavain | Flurazepam | Proflazepam | TPMPA |
| Ethanol | 7,8-Dihydro-methysticin | Flutazolam | Propanidid | ZAPA ((Z)-3-[(Aminoiminomethyl)thio]prop-2-enoic acid) |
| Etomidate | 7,8-Dihydro-yangonin | Flutemazepam | Propofol | |
| Flunitrazepam | Abecarnil | Flutoprazepam | PWZ-007A | |
| GABA | Adinazolam | Fosazepam | PWZ-009A1 | |
| Gabamide | Allobarbital | Fospropofol | Pwz-029 | |
| GABOB | Allo-pregnanolone | Ganaxolone | Pyrazolam | |
| Gaboxadol | Alphaxolone | Gbld-345 | PZ-II-028 | |

| Agonists | Modulators/Binders | | | Antagonists |
|---|---|---|---|---|
| Gamma Hydroxybutyric Acid | Alphenal | Gedocarnil | PZ-II-029 | |
| Glutethimide | Alpidem | Gidazepam | Qh-Ii-66 | |
| Ibotenic acid | Alprazolam | Girisopam | Quazepam | |
| Imidazenil | Amentoflavone | Glutethimide | Quinidine Barbiturate | |
| Isoflurane | Amobarbital | Gyki-52466 | Reclazepam | |
| Isoguvacine | Apigenin | Gyki-52895 | Remimazolam | |
| Isonipecotic acid | Aprobarbital | Halazepam | Rilmazafone | |
| Ivermectin | Arfendazam | Haloxazolam | Ripazepam | |
| L-830982 | Avizafone | Heptabarbital | Ro15-4513 | |
| Meprobamate | AZD7325 | Hexobarbital | Ro48-6791 | |
| Methoxyflurane | Baicalein | Iclazepam | Ro48-8684 | |
| MK-0777/L83098 | Baicalin | Imidazenil | Ro4938581 | |
| Methyprylon | Barbital | Indiplon | Rwj-51204 | |
| Moxidectin | Barbituric Acid Derivative | Irazepine | Saripidem | |
| Muscimol | Bentazepam | Kavain | Sarmazenil | |
| N4-Chloroacetyl-cytosine arabinoside | Brallobarbital | Kenazepine | Sb-205,384 | |
| Pagoclone | Bretazenil | Ketazolam | Scutellarein | |
| Phenibut | Bromazepam | L-655708 | Secobarbital | |
| Picamilon | Brotizolam | L-838,417 | Sevoflurane | |
| Piperazine | Butalbital | Lanthanum | Sh-053-R-Ch3-2′F | |
| Piperidine-4-sulfonic acid | Butethal | LAU 156 | Skull-capflavone II | |
| Progabide | Butobarbital | LAU 157 | Sl-651,498 | |
| QH-ii-066 | Camazepam | LAU 159 | Sodium Amytal | |
| Quisqualamine | Carburazepam | LAU 161 | Sodium Pentothal | |
| Sevoflurane | Carisoprodol | LAU 162 | Stiripentol | |
| SL 75102 | CGS 20625 | LAU 163 | Sulazepam | |
| SL-651,498 | CGS 20625 | LAU 176 | Sulfonmethane | |
| Thiamylal | CGS 8216 | LAU 177 | Suproclone | |
| Thiomuscimol | CGS 9895 | LAU 206 | Suriclone | |
| Tolgabide | CGS 9896 | Lofendazam | Sx-3228 | |
| Topiramate | Chloral Hydrate | Lopirazepam | Talampanel | |
| Zolpidem | Chloralose | Loprazolam | Talbutal | |
| α5IA | Chlordiazepoxide | Lorazepam | Taniplon | |
| | Chlormezanone | Lorbamate | Temazepam | |
| | Chloroform | Loreclezole | Tetrazepam | |

| Agonists | Modulators/Binders | | | Antagonists |
|---|---|---|---|---|
| | Ciclotizolam | Lorediplon | Tetronal | |
| | Cinazepam | Lormetazepam | Thdoc | |
| | Cinolazepam | Meclonazepam | Theanine | |
| | Cl-218,872 | Medazepam | Thiamylal | |
| | Clazolam | Menitrazepam | Thieno-diazepine | |
| | Climazolam | Mephobarbital | Thiopental | |
| | Clobazam | Meprobamate | Tofisopam | |
| | Clomethiazole | Metaclazepam | Tolufazepam | |
| | Clonazepam | Methaqualone | Tp-003 | |
| | Clonazolam | Metharbital | Tp-13 | |
| | Clorazepate | Methohexital | Tpa-023 | |
| | Clotiazepam | Methyl-phenobarbital | Triazolam | |
| | Cloxazolam | Methyprylon | Triflubazam | |
| | Cp-1414s | Methysticin | Triflunor-dazepam | |
| | CTP-354 | Metizolam | Trional | |
| | Cyclobarbital | Mexazolam | Tuclazepam | |
| | Cyprazepam | Midazolam | Uldazepam | |
| | Delorazepam | Motrazepam | Valerenic Acid | |
| | Demoxepam | N-Desalkyl-flurazepam | Valeric Acid | |
| | Deschloro-etizolam | Necopidem | Wogonin | |
| | Desmethoxy-yangonin | Nerisopam | XHe-II-006 | |
| | Desmethyl-flunitrazepam | Niacin | XHe-II-019 | |
| | Diazepam | Niacinamide | XHe-II-087c | |
| | Diclazepam | Nifoxipam | XHe-II-094 | |
| | Diethyl Ether | Nimetazepam | XHe-II-098b | |
| | Dihydro-ergotoxine | Nitrazepam | XHe-II-17 | |
| | Dihydroquinidine Barbiturate | Nitrazepate | XHe-III-006c | |
| | Diproqualone | Nitrazolam | XHe-III-063 | |
| | Divaplon | Nordazepam | XHe-III-24 | |
| | Doxefazepam | Nortetrazepam | XHe/ON-I | |
| | Elb-139 | Ns-2664 | Y-23684 | |
| | Elfazepam | Ns-2710 | Yangonin | |
| | Estazolam | Ocinaplon | Zaleplon | |
| | Eszopiclone | Oroxylin A | Zapizolam | |
| | Etaqualone | Oxazepam | Zinc | |
| | Etazepine | Oxazolam | Zk-93423 | |
| | Etazolate | Pagoclone | Zolazepam | |

| Agonists | Modulators/Binders | | | Antagonists |
|---|---|---|---|---|
| | Ethyl Carfluzepate | Panadiplon | Zolpidem | |
| | Ethyl Dirazepate | Pazinaclone | Zomebazam | |
| | Ethyl Loflazepate | PB-XHe | Zopiclone | |

**Table 4: Ligands for 5-Hydroxytryptamine Receptor (5-HT3)**

| Agonists | Modulators/ Binders | Antagonists | |
|---|---|---|---|
| 2-methyl-5-HT | 5-chloroindole | 3-Tropanyl indole-3-carboxylate | Mianserin |
| Alpha-Methyltryptamine | Trimipramine | Adr-851 | Mirisetron Maleate |
| Bufotenin | | Adr-882 | Mirtazapine |
| Chlorophenyl-biguanide | | Alosetron | Ml-1035 |
| Cisapride | | Amoxapine | Mm-218 |
| DDP733 | | Anpirtoline | N-3256 |
| Ethanol | | Aripiprazole | Netupitant |
| Ethanol | | AS-8112 | Olanzapine |
| Ibogaine | | Azasetron | Ondansetron |
| Metoclopramide | | Batanopride | Org-4419 |
| Phenylbiguanide | | Bimu-1 | Org-4419-2 |
| Quipazine | | Chloroprocaine | Palonosetron |
| RS-56812 | | Cilansetron | Pancopride |
| SR-57227 | | Clozapine | Procaine |
| Tapentadol | | Cp-81386 | Quetiapine |
| Varenicline | | Cp-93318 | R-093777 |
| YM-31636 | | Cr-3124 | R-Zacopride |
| | | DA-9701 | Ramosetron |
| | | Daizac | Renzapride |
| | | Dat-582 | Rg-12915 |
| | | Dau-6285 | Ricasetron |
| | | Ddp-225 | Rocuronium |
| | | Dolasetron | Rs-16566 |
| | | E-3620 | Rs-33800 |
| | | Fabesetron | Rs-56532 |
| | | Facinicline/RG3487 Hydrochloride | Rs-56812 |
| | | Galdansetron | S-21007 |
| | | Gastroprokinetics | Sc-50410 |
| | | Gk-128 | Sc-52150 |
| | | Gr-65630 | Sc-52246 |
| | | Granisetron | Sc-52491 |
| | | Gyki-46903 | Sc-54750 |
| | | Itasetron | Sdz-Icm-567 |

| Agonists | Modulators/ Binders | Antagonists | |
|---|---|---|---|
| | | Kb-6806 | Sep-226332 |
| | | Kf-18259 | Srss-021 |
| | | Kf-20170 | Tedatioxetine |
| | | Kga-0941 | Thujone |
| | | L-683877 | Topiramate |
| | | Lamotrigine | Tropisetron |
| | | Lerisetron | Tubocurarine |
| | | Lintopride | Tzb-30878 |
| | | Litoxetine | Va-21B7 |
| | | Loxapine | Vi-0134 |
| | | Lurosetron | Vortioxetine |
| | | Ly-278584 | Vortioxetine Hydrobromide |
| | | M1, the major active metabolite of mosapride | Way-100289 |
| | | Mci-225 | Ym-114 |
| | | Mci-225 | Ym-26103-2 |
| | | Memantine | Ym-26308-2 |
| | | Menthol | Zacopride |
| | | Methadone | Zatosetron |
| | | Metoclopramide | Ziprasidone |

**Table 5: Ligands for Nicotinic Acetylcholine Receptor (nAchR)**

| Agonists | Modulators/Binders | Antagonists |
|---|---|---|
| (+)-N-(1-azabicyclo[2.2.2]oct-3-yl)benzo[b]furan- 2-carboxamide | A-867744 | (−)-7-methyl-2-exo-[3'-(6-[18F]fluoropyridin-2-yl)-5'-pyridinyl]-7-azabicyclo[2.2.1]heptane |
| 3-Bromocytisine | AQW-051 | 2-fluoro-3-(4-nitro-phenyl)deschloroepibatidine |
| A-366,833 | AVL-3288 | Acv-1 |
| A-582941 | Desformylflustrabromine | ACVx |
| A-82695 | Ethanol | Amobarbital |
| A-84,543 | Galantamine | Anandamide |
| ABT-089 | Ivermectin | Anq-9040 |
| ABT-126 | Nefiracetam | Aprobarbital |
| Abt-202 | NS-1738 | ATG-001 |
| ABT-418 | NS-9283 | ATG003 |
| Abt-418 | PNU-120,596 | Atracurium besylate |
| Abt-560 | Tetraethylammonium | Barbital |
| ABT-894 | | Barbituric acid derivative |
| Acetylcholine | | Biperiden |
| Altinicline | | Bupropion |
| Altinicline | | Butabarbital |

| Agonists | Modulators/Binders | Antagonists |
|---|---|---|
| Aminoethoxypyridine | | Butalbital |
| Anabasine | | Butethal |
| AR-R17779 | | Chloroprocaine |
| Asm-024 | | Cisatracurium Besilate |
| AZD-0328 | | Cisatracurium besylate |
| Bupropion Hydrochloride | | Coclaurine |
| Carbachol | | Dehydronorketamine |
| Choline | | Deuterated Bupropion |
| Cm-2433 | | Dextromethorphan |
| CP-601927 | | Doxacurium chloride |
| CP-601932 | | Ethanol |
| Cp-810123 | | Gallamine Triethiodide |
| Cytisine | | Heptabarbital |
| DBO-83 | | Hexobarbital |
| Decamethonium | | Hydroxybupropion |
| Dianicline | | Hydroxynorketamine |
| Dianicline | | Inaperisone |
| Encenicline | | Iptakalim |
| Epibatidine | | Isoflurane |
| EVP-6124 | | Ketamine |
| Evp-6124 | | Kynurenic acid |
| Galantamine | | Levomethadyl Acetate |
| GTS-21 | | Lobeline |
| Gts-21 | | Mecamylamine |
| ICH-3 | | Mecamylamine |
| Ispronicline | | Memantine |
| Levamisole | | Methadone |
| Lobeline | | Metharbital |
| Lobeline Sulphate | | Methyllycaconitine |
| Mem-3454 | | Methylphenobarbital |
| MEM-63908 | | Metocurine |
| Mem-63908 | | Metocurine Iodide |
| N-(3-pyridinyl)-bridged bicyclic diamine | | Mivacurium |
| Nicotine | | Neramexane |
| PH-399733 | | Nic-002 |
| Ph-399733 | | Nitrous oxide |
| PHA-543,613 | | Norketamine |
| Pha-543613 | | Org-9991 |
| PHA-709829 | | Pancuronium |
| PNU-282,987 | | Pentobarbital |

| Agonists | Modulators/Binders | Antagonists |
|---|---|---|
| Pnu-282987 | | Pentolinium |
| Pozanicline | | Phenobarbital |
| Pozanicline | | Pipecuronium |
| Rivanicline | | Pipecuronium |
| Rivanicline | | PNU-120,596 |
| Rjr-1401 | | Primidone |
| Sar-130479 | | Procaine |
| Sazetidine A | | Quinolizidine (–)-1-epi-207I |
| Sib-1663 | | RJR-2531 |
| Sib-1765f | | Rocuronium |
| Sib-3182 | | RPI-78M |
| Sofinicline | | Secobarbital |
| SSR-180,711 | | Talbutal |
| Succinylcholine | | Thiopental |
| Suvn-F90101 | | Trimethaphan |
| Suvn-F91201 | | Tubocurarine |
| Tacrine-huperzine A | | U-2902 |
| TC-1698 | | Vecuronium |
| TC-1827 | | α-Bungarotoxin |
| Tc-2216 | | α-Conotoxin |
| Tc-2403 | | |
| Tc-2429 | | |
| Tc-2559 | | |
| Tc-2696 | | |
| TC-5619 | | |
| Tebanicline | | |
| Tropisetron | | |
| Ub-165 | | |
| Varenicline | | |
| WAY-317,538 | | |
| CNL001 | | |
| CNL002 | | |

**Table 6: Ligands for ATP-Gated P2X Receptor Cation Channel (P2X)**

| Agonists | Modulators /Binders | Antagonists | |
|---|---|---|---|
| ATP | Ivermectin | 4-benzoyl-1-substituted-piperazin-2-ones | MK-801 |
| BzATP | | 5-methyl-6,7-dihydro-5Hcyclopentapyrazine | MRS2159 |
| α,β-meATP | | 5-oxo-3-pyrrolidinecarboxamides | NF023 |
| | | 5,6,7,8-tetrahydropyrido[4,3,d]-pyrimidines | NF279 |

| Agonists | Modulators /Binders | Antagonists | |
|---|---|---|---|
| | | A-317491 | NF449 |
| | | Amitriptyline | Nortriptyline |
| | | Azaindole-3-carboxamides | Oxoisoquinoline carboxamides |
| | | AZD-9056 | Paroxetine |
| | | Benzamide 6 | Piperazines |
| | | Benzofuro-1,4-diazepin-2-ones | Polycyclic guanines |
| | | Benzoimidazoles | PPADS |
| | | Biaryl benzamides | PPNDS |
| | | Carboxamides | Pyrazolo-[1,5,a]-pyridine carboxamides |
| | | CE-224535 | Pyridazinones |
| | | Desipramine | Pyridoxal-5-phosphate |
| | | Diaminopyridines | Pyrrolinones |
| | | Doxepin | Pyrrolo-[2,3,b]-pyridine carboxamides |
| | | Eslicarbazepine acetate | Pyrrolopyrimidin-7-ones |
| | | EVT-401 | Quinoline carboxamides |
| | | Fluoxetine | RO-3 |
| | | Hyaluronic acid derivatives | RO-4 |
| | | Imipramine | RO-51 |
| | | Indole carboxamides | Spinorphins |
| | | Indole-3-carboxamides | Suramin |
| | | Ip5I | Tetrahydro-2H-1,2-thiazine 1,1-dioxides |
| | | Isoquinoline carboxamides | TNP-ATP |
| | | Isothiazolidine 1,1-dioxides | |

**Table 7: Ligands for Inwardly Rectifying Potassium Channel (Kir)**

| Agonists | Modulators / Binders | Antagonists |
|---|---|---|
| Diazoxide | | Acetohexamide |
| Iptakalim | | Carbutamide |
| Minoxidil | | Chlorpropamide |
| Nicorandil | | Glibenclamide |
| Phosphatidylinositol 4,5-Bisphosphate | | Glibenclamide |
| Pinacidil | | Glibornuride |
| | | Gliclazide |
| | | Glimepiride |
| | | Glipizide |
| | | Gliquidone |
| | | Glisoxepide |
| | | Glyburide |
| | | Glyclopyramide |

| Agonists | Modulators / Binders | Antagonists |
|---|---|---|
| | | Glycyclamide |
| | | Metahexamide |
| | | Tolazamide |
| | | Tolbutamide |
| | | Tolhexamide |

**Table 8: Ligands for Voltage Dependent Potassium Channel (KCNQ/Kv7)**

| Agonists | Modulators / Binders | Antagonists |
|---|---|---|
| Diazoxide | | Azimilide |
| Flupirtine | | Amiodarone |
| Minoxidil | | Bretylium |
| Nicorandil | | Clofilium |
| Pinacidil | | Dalfampridine |
| Retigabine | | Dofetilide |
| | | E-4031 |
| | | Ibutilide |
| | | Nifekalant |
| | | Sematilide |
| | | Sotalol |
| | | Sulfonylureas |
| | | Tedisamil |

**Table 9: Ligands for Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)**

| Agonists | Modulators / Binders | Antagonists |
|---|---|---|
| | 8-cyclopentyl-1,3-dipropylxanthine | Bumetanide |
| | 8-methoxypsoralen | Crofelemer |
| | Apigenin | Glyburide |
| | CTP-656 | Ibuprofen |
| | Genistein | |
| | IBMX | |
| | Ivacaftor | |
| | Lumacaftor | |

## E. Polynucleotides

**[0226]** In various illustrative embodiments, the present disclosure contemplates, in part, polynucleotides, polynucleotides encoding engineered receptor polypeptides including LGICs, and subunits and muteins thereof, and fusion polypeptides, viral vector polynucleotides, and compositions comprising the same.

**[0227]** As used herein, the terms “polynucleotide,” “nucleotide,” “nucleotide sequence” or “nucleic acid” are used interchangeably. They refer to a polymeric form of

nucleotides of any length, either deoxyribonucleotides or ribonucleotides, or analogs thereof. Polynucleotides may have any three dimensional structure, and may perform any function, known or unknown. The following are non-limiting examples of polynucleotides: coding or non-coding regions of a gene or gene fragment, loci (locus) defined from linkage analysis, exons, introns, messenger RNA (mRNA), transfer RNA (tRNA), ribosomal RNA (rRNA), short interfering RNA (siRNA), short-hairpin RNA (shRNA), micro-RNA (miRNA), ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes, and primers. A polynucleotide may comprise one or more modified nucleotides, such as methylated nucleotides and nucleotide analogs. If present, modifications to the nucleotide structure may be imparted before or after assembly of the polymer. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may be further modified after polymerization, such as by conjugation with a labeling component. Polynucleotides may be deoxyribonucleic acid (DNA), ribonucleic acid (RNA) or DNA/RNA hybrids. Polynucleotides may be single-stranded or double-stranded. Polynucleotides include, but are not limited to: pre-messenger RNA (pre-mRNA), messenger RNA (mRNA), RNA, short interfering RNA (siRNA), short hairpin RNA (shRNA), microRNA (miRNA), ribozymes, synthetic RNA, genomic RNA (gRNA), plus strand RNA (RNA(+)), minus strand RNA (RNA(-)), synthetic RNA, genomic DNA (gDNA), PCR amplified DNA, complementary DNA (cDNA), synthetic DNA, or recombinant DNA. Polynucleotides refer to a polymeric form of nucleotides of at least 5, at least 10, at least 15, at least 20, at least 25, at least 30, at least 40, at least 50, at least 100, at least 200, at least 300, at least 400, at least 500, at least 1000, at least 5000, at least 10000, or at least 15000 (including all ranges and subranges therebetween) or more nucleotides in length, either ribonucleotides or deoxynucleotides or a modified form of either type of nucleotide, as well as all intermediate lengths. It will be readily understood that "intermediate lengths" in this context, means any length between the quoted values, such as 6, 7, 8, 9, *etc.*, 101, 102, 103, *etc.*; 151, 152, 153, *etc.*; 201, 202, 203, *etc.* In particular embodiments, polynucleotides or variants have at least or about 50%, 55%, 60%, 65%, 70%, 71%, 72%, 73%, 74%, 75%,76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%,85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% (including all ranges and subranges therebetween) sequence identity to a reference sequence described herein or known in the art, typically where the variant maintains at least one biological activity of the reference sequence unless otherwise stated.

**[0228]** As used herein, the term “gene” may refer to a polynucleotide sequence comprising enhancers, promoters, introns, exons, and the like. In particular embodiments, the term “gene” refers to a polynucleotide sequence encoding a polypeptide, regardless of whether the polynucleotide sequence is identical to the genomic sequence encoding the polypeptide.

**[0229]** As used herein, a “cis-acting sequence, “cis-acting regulatory sequence”, or “cis-acting nucleotide sequence” or equivalents refers to a polynucleotide sequence that is associated with the expression, e.g. transcription and/or translation, of a gene. In one embodiment, the cis-acting sequence regulates transcription because it is a binding site for a polypeptide that represses or decreases transcription or a polynucleotide sequence associated with a transcription factor binding site that contributes to transcriptional repression. Examples of cis-acting sequences that regulate the expression of polynucleotide sequences and that may be operably linked to the polynucleotides of the present disclosure to regulate the expression of the subject engineered receptors are well known in the art and include such elements as promoter sequences (e.g CAG, CMV, SYN, CamKII, TRPV1), Kozak sequences, enhancers, posttranscriptional regulatory elements, miRNA binding elements, and polyadenylation sequences.

**[0230]** As one non-limiting example, a promoter sequence is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. For purposes of defining the present invention, the promoter sequence is bounded at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence will be found a transcription initiation site, as well as protein binding domains responsible for the binding of RNA polymerase. Eukaryotic promoters will often, but not always, contain "TATA" boxes and "CAT" boxes. Various promoters may be used to drive the various vectors of the present invention. For example, the promoter may be a constitutively active promoter, i.e. a promoter that is active in the absence externally applied ligands, e.g. the CMV IE1 promoter, the SV40 promoter, GAPDH promoter, Actin promoter. The promoter may be an inducible promoter, i.e. a promoter whose activity is regulated upon the application of an ligand to the cell, e.g. doxycycline, the tet-on or tet-off promoter, the estrogen receptor promoter, etc. The promoter may be a tissue-specific promoter, i.e. a promoter that is active on certain types of cells.

**[0231]** In some embodiments, the promoter is active in an excitable cell. By an “excitable cell”, it is meant a cell that is activated by a change in membrane potential, e.g. a

neuron or myocyte, e.g. a dorsal root ganglion neuron, a motor neuron, an excitatory neuron, an inhibitory neuron, or a sensory neuron. Promoters that are active in an excitable cell that would find use in the present polynucleotide compositions would include neuronal promoters, for example, the synapsin (SYN), TRPV1, $Na_v1.7$, $Na_v1.8$, $Na_v1.9$, CamKII, NSE, and Advillin promoters; myocyte promoters, e.g. the desmin (Des), alpha-myosin heavy chain (α-MHC), myosin light chain 2 (MLC-2) and cardiac troponin C (cTnC) promoters; and ubiquitous acting promoters, e.g. CAG, CBA, E1Fa, Ubc, CMV, and SV40 promoters.

**[0232]** As used herein, a "regulatory element for inducible expression" refers to a polynucleotide sequence that is a promoter, enhancer, or functional fragment thereof that is operably linked to a polynucleotide to be expressed and that responds to the presence or absence of a molecule that binds the element to increase (turn-on) or decrease (turn-off) the expression of the polynucleotide operably linked thereto. Illustrative regulatory elements for inducible expression include, but are not limited to, a tetracycline responsive promoter, an ecdysone responsive promoter, a cumate responsive promoter, a glucocorticoid responsive promoter, an estrogen responsive promoter, an RU-486 responsive promoter, a PPAR-γ promoter, and a peroxide inducible promoter.

**[0233]** A "regulatory element for transient expression" refers to a polynucleotide sequence that can be used to briefly or temporarily express a polynucleotide nucleotide sequence. In particular embodiments, one or more regulatory elements for transient expression can be used to limit the duration of a polynucleotide. In certain embodiments, the preferred duration of polynucleotide expression is on the order of minutes, hours, or days. Illustrative regulatory elements for transient expression include, but are not limited to, nuclease target sites, recombinase recognition sites, and inhibitory RNA target sites. In addition, to some extent, in particular embodiments, a regulatory element for inducible expression may also contribute to controlling the duration of polynucleotide expression.

**[0234]** As used herein, the terms "polynucleotide variant" and "variant" and the like refer to polynucleotides displaying substantial sequence identity with a reference polynucleotide sequence or polynucleotides that hybridize with a reference sequence under stringent conditions that are defined hereinafter. These terms also encompass polynucleotides that are distinguished from a reference polynucleotide by the addition, deletion, substitution, or modification of at least one nucleotide. Accordingly, the terms "polynucleotide variant" and "variant" include polynucleotides in which one or more nucleotides have been added or deleted, or modified, or replaced with different nucleotides. In this regard, it is well understood

in the art that certain alterations inclusive of mutations, additions, deletions and substitutions can be made to a reference polynucleotide whereby the altered polynucleotide retains the biological function or activity of the reference polynucleotide. In particular embodiments, polynucleotides or variants have at least or about 50%, 55%, 60%, 65%, 70%, 71%, 72%, 73%, 74%, 75%,76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%,85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% (including all ranges and subranges therebetween) sequence identity to a reference sequence described herein or known in the art, typically where the variant maintains at least one biological activity of the reference sequence unless otherwise stated.

**[0235]** In one embodiment, a polynucleotide comprises a nucleotide sequence that hybridizes to a target nucleic acid sequence under stringent conditions. To hybridize under "stringent conditions" describes hybridization protocols in which nucleotide sequences at least 60% identical to each other remain hybridized. Generally, stringent conditions are selected to be about 5ºC lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength, pH and nucleic acid concentration) at which 50% of the probes complementary to the target sequence hybridize to the target sequence at equilibrium. Since the target sequences are generally present at excess, at Tm, 50% of the probes are occupied at equilibrium.

**[0236]** The recitations "sequence identity" or, for example, comprising a "sequence 50% identical to," as used herein, refer to the extent that sequences are identical on a nucleotide-by-nucleotide basis or an amino acid-by-amino acid basis over a window of comparison. Thus, a "percentage of sequence identity" may be calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (*e.g.*, A, T, C, G, I) or the identical amino acid residue (*e.g.*, Ala, Pro, Ser, Thr, Gly, Val, Leu, Ile, Phe, Tyr, Trp, Lys, Arg, His, Asp, Glu, Asn, Gln, Cys and Met) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (*i.e.*, the window size), and multiplying the result by 100 to yield the percentage of sequence identity. Terms used to describe sequence relationships between two or more polynucleotides or polypeptides include "reference sequence," "comparison window," "sequence identity," "percentage of sequence identity," and "substantial identity." A "reference sequence" is at least 12 but frequently 15 to 18 and often at least 25 monomer units, inclusive of nucleotides and amino acid residues, in length. Because two polynucleotides may each comprise (1) a sequence (*i.e.*, only a portion of the complete polynucleotide sequence) that is

similar between the two polynucleotides, and (2) a sequence that is divergent between the two polynucleotides, sequence comparisons between two (or more) polynucleotides are typically performed by comparing sequences of the two polynucleotides over a "comparison window" to identify and compare local regions of sequence similarity. A "comparison window" refers to a conceptual segment of at least 6 contiguous positions, usually about 50 to about 100, more usually about 100 to about 150 in which a sequence is compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. The comparison window may comprise additions or deletions (*i.e.*, gaps) of about 20% or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Optimal alignment of sequences for aligning a comparison window may be conducted by computerized implementations of algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Drive Madison, WI, USA) or by inspection and the best alignment (*i.e.*, resulting in the highest percentage homology over the comparison window) generated by any of the various methods selected. Reference also may be made to the BLAST family of programs as for example disclosed by Altschul *et al.,* 1997, *Nucl. Acids Res.* 25:3389. A detailed discussion of sequence analysis can be found in Unit 19.3 of Ausubel *et al.*, *Current Protocols in Molecular Biology*, John Wiley & Sons Inc, 1994-1998, Chapter 15.

**[0237]** An "isolated polynucleotide," as used herein, refers to a polynucleotide that has been purified from the sequences which flank it in a naturally-occurring state, *e.g.,* a DNA fragment that has been removed from the sequences that are normally adjacent to the fragment. In particular embodiments, an "isolated polynucleotide" refers to a complementary DNA (cDNA), a recombinant DNA, or other polynucleotide that does not exist in nature and that has been made by the hand of man.

**[0238]** Terms that describe the orientation of polynucleotides include: 5' (normally the end of the polynucleotide having a free phosphate group) and 3' (normally the end of the polynucleotide having a free hydroxyl (OH) group). Polynucleotide sequences can be annotated in the 5' to 3' orientation or the 3' to 5' orientation. For DNA and mRNA, the 5' to 3' strand is designated the "sense," "plus," or "coding" strand because its sequence is identical to the sequence of the pre-messenger (premRNA) [except for uracil (U) in RNA, instead of thymine (T) in DNA]. For DNA and mRNA, the complementary 3' to 5' strand which is the strand transcribed by the RNA polymerase is designated as "template," "antisense," "minus," or "non-coding" strand. As used herein, the term "reverse orientation" refers to a 5' to 3'

sequence written in the 3' to 5' orientation or a 3' to 5' sequence written in the 5' to 3' orientation.

**[0239]** The term "flanked" refers to a polynucleotide sequence that is in between an upstream polynucleotide sequence and/or a downstream polynucleotide sequence, *i.e.*, 5' and/or 3', relative to the sequence. For example, a sequence that is "flanked" by two other elements (*e.g.*, ITRs), indicates that one element is located 5' to the sequence and the other is located 3' to the sequence; however, there may be intervening sequences therebetween.

**[0240]** The terms "complementary" and "complementarity" refer to polynucleotides (*i.e.*, a sequence of nucleotides) related by the base-pairing rules. For example, the complementary strand of the DNA sequence 5' A G T C A T G 3' is 3' T C A G T A C 5'. The latter sequence is often written as the reverse complement with the 5' end on the left and the 3' end on the right, 5' C A T G A C T 3'. A sequence that is equal to its reverse complement is said to be a palindromic sequence. Complementarity can be "partial," in which only some of the nucleic acids' bases are matched according to the base pairing rules. Or, there can be "complete" or "total" complementarity between the nucleic acids.

**[0241]** The terms "nucleic acid cassette" or "expression cassette" as used herein refers to polynucleotide sequences within a larger polynucleotide, such as a vector, which are sufficient to express one or more RNAs from a polynucleotide. The expressed RNAs may be translated into proteins, may function as guide RNAs or inhibitory RNAs to target other polynucleotide sequences for cleavage and/or degradation. In one embodiment, the nucleic acid cassette contains one or more polynucleotide(s)-of-interest. In another embodiment, the nucleic acid cassette contains one or more expression control sequences operably linked to one or more polynucleotide(s)-of-interest. Polynucleotides include polynucleotide(s)-of-interest. As used herein, the term "polynucleotide-of-interest" refers to a polynucleotide encoding a polypeptide or fusion polypeptide or a polynucleotide that serves as a template for the transcription of an inhibitory polynucleotide, *e.g.*, LGICs, and subunits and muteins thereof, as contemplated herein. In a particular embodiment, a polynucleotide-of-interest encodes a polypeptide or fusion polypeptide having one or more enzymatic activities, such as a nuclease activity and/or chromatin remodeling or epigenetic modification activities.

**[0242]** Vectors may comprise 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more nucleic acid cassettes. In a preferred embodiment of the disclosure, a nucleic acid cassette comprises one or more expression control sequences (*e.g.*, a promoter or enhancer operable in a neuronal cell) operably linked to a polynucleotide encoding a engineered receptor, *e.g.*, an LGIC, or subunit

or muteins thereof. The cassette can be removed from or inserted into other polynucleotide sequences, *e.g.*, a plasmid or viral vector, as a single unit.

**[0243]** In one embodiment, a polynucleotide contemplated herein comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, or more nucleic acid cassettes any number or combination of which may be in the same or opposite orientations.

**[0244]** Moreover, it will be appreciated by those of ordinary skill in the art that, as a result of the degeneracy of the genetic code, there are many nucleotide sequences that may encode a polypeptide, or fragment of variant thereof, as contemplated herein. Some of these polynucleotides bear minimal homology to the nucleotide sequence of any native gene. Nonetheless, polynucleotides that vary due to differences in codon usage are specifically contemplated by the present disclosure, for example polynucleotides that are optimized for human and/or primate codon selection. In one embodiment, polynucleotides comprising particular allelic sequences are provided. Alleles are endogenous polynucleotide sequences that are altered as a result of one or more mutations, such as deletions, additions and/or substitutions of nucleotides.

**[0245]** In some embodiments, the present disclosure provides a polynucleotide encoding an engineered receptor described herein. In some embodiments, the present disclosure provides a polynucleotide encoding a chimeric engineered LGIC receptor described herein.

**[0246]** In some embodiments, the present disclosure provides a polynucleotide encoding an engineered receptor comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of one of SEQ ID NOs: 89-98. In some embodiments, the present disclosure provides a polynucleotide encoding an engineered receptor comprising an amino acid sequence of one of SEQ ID NOs: 89-98. In some embodiments, the present disclosure provides a polynucleotide encoding an engineered receptor consisting of an amino acid sequence of one of SEQ ID NOs: 89-98.

## F. Vectors

**[0247]** In some aspects of the disclosure, a nucleic acid molecule, i.e., a polynucleotide encoding an engineered receptor is delivered to a subject. In some cases, the nucleic acid molecule encoding the engineered receptor is delivered to a subject by a vector. In various embodiments, a vector comprises a one or more polynucleotide sequences contemplated herein. The term “vector” is used herein to refer to a nucleic acid molecule capable of transferring or

transporting another nucleic acid molecule. The transferred polynucleotide is generally linked to, *e.g.*, inserted into, the vector nucleic acid molecule. A vector may include sequences that direct autonomous replication in a cell, or may include sequences sufficient to allow integration into host cell DNA. A vector can deliver a target polynucleotide to an organism, a cell or a cellular component. In some cases, the vector is an expression vector. An "expression vector" as used herein refers to a vector, for example, a plasmid, that is capable of promoting expression, as well as replication of a polynucleotide incorporated therein. Typically, the nucleic acid sequence to be expressed is operably linked to cis-acting regulatory sequence, e.g. a promoter and/or enhancer sequence, and is subject to transcription regulatory control by the promoter and/or enhancer. In particular cases, a vector is used to deliver a nucleic acid molecule encoding an engineered receptor of the disclosure to a subject.

**[0248]** In particular embodiments, any vector suitable for introducing an expression cassette or polynucleotide encoding an engineered receptor into a neuronal cell can be employed. Illustrative examples of suitable vectors include plasmids (*e.g.*, DNA plasmids or RNA plasmids), transposons, cosmids, bacterial artificial chromosomes, and viral vectors. In some cases, the vector is a circular nucleic acid, for *e.g.*, a plasmid, a BAC, a PAC, a YAC, a cosmid, a fosmid, and the like. In some cases, circular nucleic acid molecules can be utilized to deliver a nucleic acid molecule encoding an engineered receptor to a subject. For example, a plasmid DNA molecule encoding an engineered receptor can be introduced into a cell of a subject whereby the DNA sequence encoding the engineered receptor is transcribed into mRNA and the mRNA "message" is translated into a protein product. The circular nucleic acid vector will generally include regulatory elements that regulate the expression of the target protein. For example, the circular nucleic acid vector may include any number of promoters, enhancers, terminators, splice signals, origins of replication, initiation signals, and the like.

**[0249]** In some cases, the vector can include a replicon. A replicon may be any nucleic acid molecule capable of self-replication. In some cases, the replicon is an RNA replicon derived from a virus. A variety of suitable viruses (*e.g.* RNA viruses) are available, including, but not limited to, alphavirus, picornavirus, flavivirus, coronavirus, pestivirus, rubivirus, calcivirus, and hepacivirus.

**[0250]** In some embodiments, the vector is a non-viral vector. By a "non-viral vector", it is meant any delivery vehicle that does not comprise a viral capsid or envelope, *e.g.* lipid nanoparticles (anionic (negatively charged), neutral, or cationic (positively charged)), heavy metal nanoparticles, polymer-based particles, plasmid DNA, minicircle DNA, minivector DNA, ccDNA, synthetic RNA, exosomes, and the like. Non-viral vectors may be delivered by

any suitable method as would be well understood in the art, including, *e.g.*, nanoparticle delivery, particle bombardment, electroporation, sonication, or microinjection. See, *e.g.* Chen et al. Mol. Therapy, Methods and Clinical Development. 2016 Jan; Vol 3, issue 1; and Hardy, CE et al. Genes (Basel). 2017 Feb; 8(2): 65

**[0251]** In other embodiments, the vector is a viral vector. By a "viral vector" it is meant a delivery vehicle that comprises a viral capsid or envelop surrounding a polynucleotide encoding an RNA or polypeptide of interest. In some cases, the viral vector is derived from a replication-deficient virus. Non-limiting examples of viral vectors suitable for delivering a nucleic acid molecule of the disclosure to a subject include those derived from adenovirus, retrovirus (*e.g.*, lentivirus), adeno-associated virus (AAV), and herpes simplex-1 (HSV-1). Illustrative examples of suitable viral vectors include, but are not limited to, retroviral vectors (*e.g.*, lentiviral vectors), herpes virus based vectors and parvovirus based vectors (*e.g.*, adeno-associated virus (AAV) based vectors, AAV-adenoviral chimeric vectors, and adenovirus-based vectors).

**[0252]** The term "parvovirus" as used herein encompasses all parvoviruses, including autonomously-replicating parvoviruses and dependoviruses. The autonomous parvoviruses include members of the genera *Parvovirus, Erythrovirus, Densovirus, Iteravirus*, and *Contravirus*. Exemplary autonomous parvoviruses include, but are not limited to, mouse minute virus, bovine parvovirus, canine parvovirus, chicken parvovirus, feline panleukopenia virus, feline parvovirus, goose parvovirus, and B19 virus. Other autonomous parvoviruses are known to those skilled in the art. *See, e.g.*, Fields *et al.*, 1996 *Virology*, volume 2, chapter 69 (3d ed., Lippincott-Raven Publishers).

**[0253]** The genus *Dependovirus* contains the adeno-associated viruses (AAV), including but not limited to, AAV type 1, AAV type 2, AAV type 3, AAV type 4, AAV type 5, AAV type 6, AAV type 7, AAV type 8, AAV type 9, AAV type rh10, avian AAV, bovine AAV, canine AAV, equine AAV, and ovine AAV.

**[0254]** In a preferred embodiment, the vector is an AAV vector. In particular cases, the viral vector is an AAV5, AAV-6 or AAV-9 vector.

**[0255]** The genomic organization of all known AAV serotypes is similar. The genome of AAV is a linear, single-stranded DNA molecule that is less than about 5,000 nucleotides (nt) in length. Inverted terminal repeats (ITRs) flank the unique coding nucleotide sequences for the non-structural replication (Rep) proteins and the structural (VP) proteins. The VP proteins (VPl, -2 and -3) form the capsid and contribute to the tropism of the virus. The terminal 145 nt ITRs are self-complementary and are organized so that an energetically stable intramolecular

duplex forming a T-shaped hairpin may be formed. These hairpin structures function as an origin for viral DNA replication, serving as primers for the cellular DNA polymerase complex. Following wild-type (wt) AAV infection in mammalian cells the Rep genes are expressed and function in the replication of the viral genome.

**[0256]** In some cases, the outer protein "capsid" of the viral vector occurs in nature, *e.g.* AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, or AAV10. In particular cases, the capsid is synthetically engineered (*e.g.* through directed evolution or rational design) to possess certain unique characteristics not present in nature such as altered tropism, increased transduction efficiency, or immune evasion. An example of a rationally designed capsid is the mutation of one or more surface-exposed tyrosine (Y), serine (S), threonine (T), and lysine (K) residues on the VP3 viral capsid protein. Non-limiting examples of viral vectors whose VP3 capsid proteins have been synthetically engineered and are amenable for use with the compositions and methods provided herein include: AAV1(Y705+731F+T492V), AAV2(Y444+500+730F+T491V), AAV3(Y705+731F), AAV5(Y436+693+719F), AAV6(Y705+731F+T492V), AAV8(Y733F), AAV9(Y731F), and AAV10(Y733F). Non-limiting examples of viral vectors that have been engineered through directed evolution and are amenable for use with the compositions and methods provided herein include AAV-7m8 and AAV-ShH10. In some embodiments, the viral vector comprises an AAV capsid protein comprising amino acid mutation at one or more positions corresponding to T492, Y705, Y731, or any combination thereof, of AAV6 capsid protein, wherein the AAV capsid protein is of serotype AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, or another AAV serotype. In some embodiments, the one or more positions are two or more positions, two positions, or three positions. In some embodiments, the viral vector comprises an AAV capsid protein comprising one or more amino acid substitutions corresponding to T492V, Y705F or Y731F, or any combination thereof, of AAV6 capsid protein, wherein the AAV capsid protein is of serotype AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, or another AAV serotype. In some embodiments, the one or more substitutions are two or more substitutions, two substitutions, or three substitutions.

**[0257]** In some embodiments, the viral vector comprises an AAV5 capsid protein comprising a mutation at one or more amino acid positions selected from Y693 and Y719. In some embodiments, the viral vector comprises an AAV5 capsid protein comprising one or more mutations selected from Y693F and Y719F. In some embodiments, the viral vector comprises an AAV5 capsid protein comprising the amino acid mutation Y693F+Y719F. In

some embodiments, the AAV5 capsid protein comprises or consists of an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100 % sequence identity to SEQ ID NO: 79.

**[0258]** In some embodiments, the viral vector comprises an AAV6 capsid protein comprising a mutation at one or more amino acid positions selected from T492, Y705 and Y731. In some embodiments, the viral vector comprises an AAV6 capsid protein comprising one or more mutations selected from T492V, Y705F, and Y731F. In some embodiments, the viral vector comprises an AAV6 capsid protein comprising the amino acid mutation T492V+Y705F+Y731F. In some embodiments, the AAV6 capsid protein comprises or consists of an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100 % sequence identity to SEQ ID NO: 80.

**[0259]** In some embodiments, the viral vector comprises an AAV9 capsid protein comprising a mutation at one or more amino acid positions selected from T492, Y705 and Y731. In some embodiments, the viral vector comprises an AAV9 capsid protein comprising one or more mutations selected from T492V, Y705F, and Y731F. In some embodiments, the viral vector comprises an AAV9 capsid protein comprising the amino acid mutation T492V+Y705F+Y731F. In some embodiments, the AAV9 capsid protein comprises or consists of an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100 % sequence identity to SEQ ID NO: 81.

**[0260]** In some embodiments, the viral vector is an AAV9 vector, or a variant thereof. In some embodiments, the capsid polypeptide of the AAV9 vector comprises, consists essentially of, or consists of a sequence according to SEQ ID NO: 81. In some embodiments, the capsid polypeptide of the disclosure comprises, consists essentially of, or consists of an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% sequence identity to SEQ ID NO: 81. In some embodiments, the capsid polypeptide of the disclosure comprises, consists essentially of, or consists of an amino acid sequence having up to 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids mutated (substituted, deleted and/or added) as compared to the sequence of SEQ ID NO: 81. In some embodiments, the capsid polypeptide of the AAV9 vector comprises, consists essentially of, or consists of an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 99.7% sequence identity to SEQ ID NO: 81, wherein the capsid polypeptide comprises an amino acid substitution at the position corresponding to T492 of SEQ ID NO: 81. In some embodiments, the capsid polypeptide of the disclosure comprises, consists essentially of, or

consists of an amino acid sequence having up to 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids mutated (substituted, deleted and/or added) as compared to the sequence of SEQ ID NO: 81, wherein the capsid polypeptide comprises an amino acid substitution at the position corresponding to T492 of SEQ ID NO: 81. In some embodiments, the substitution is T492V. In some embodiments, the substitution is T492I. In some embodiments, the substitution is T492L. In some embodiments, the T492 residue is substituted by a hydrophobic amino acid selected from valine (Val), leucine (Leu), and isoleucine (Ile). In some embodiments, the T492 residue is substituted by a hydrophobic amino acid selected from glycine (Gly), alanine (Ala), valine (Val), leucine (Leu), isoleucine (Ile), proline (Pro), phenylalanine (Phe), methionine (Met), and tryptophan (Trp).

**[0261]** In some embodiments, the viral vector comprising the AAV capsid protein (or a mutant of the disclosure) contributes to targeted expression of an engineered receptor to a sub-population of cells or neurons in a subject. In some embodiments, the neurons are nociceptors.

**[0262]** A "recombinant parvoviral or AAV vector" (or "rAAV vector") herein refers to a vector comprising one or more polynucleotides contemplated herein that are flanked by one or more AAV ITRs. Such polynucleotides are said to be "heterologous" to the ITRs, as such combinations do not ordinarily occur in nature. Such rAAV vectors can be replicated and packaged into infectious viral particles when present in an insect host cell that is expressing AAV rep and cap gene products (*i.e.*, AAV Rep and Cap proteins). When an rAAV vector is incorporated into a larger nucleic acid construct (*e.g.*, in a chromosome or in another vector such as a plasmid or baculovirus used for cloning or transfection), then the rAAV vector is typically referred to as a "pro-vector" which can be "rescued" by replication and encapsidation in the presence of AAV packaging functions and necessary helper functions.

**[0263]** In particular embodiments, any AAV ITR may be used in the AAV vectors, including ITRs from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV13, AAV14, AAV15, and AAV16. In one preferred embodiment, an AAV vector contemplated herein comprises one or more AAV2 ITRs.

**[0264]** rAAV vectors comprising two ITRs have a payload capacity of about 4.4 kB. Self-complementary rAAV vectors contain a third ITR and package two strands of the recombinant portion of the vector leaving only about 2.1 kB for the polynucleotides contemplated herein. In one embodiment, the AAV vector is an scAAV vector.

**[0265]** Extended packaging capacities that are roughly double the packaging capacity of an rAAV (about 9kB) have been achieved using dual rAAV vector strategies. Dual vector

strategies useful in producing rAAV contemplated herein include, but are not limited to splicing (trans-splicing), homologous recombination (overlapping), or a combination of the two (hybrid). In the dual AAV trans-splicing strategy, a splice donor (SD) signal is placed at the 3′ end of the 5′-half vector and a splice acceptor (SA) signal is placed at the 5′ end of the 3′-half vector. Upon co-infection of the same cell by the dual AAV vectors and inverted terminal repeat (ITR)-mediated head-to-tail concatemerization of the two halves, trans-splicing results in the production of a mature mRNA and full-size protein (Yan *et al.*, 2000). Trans-splicing has been successfully used to express large genes in muscle and retina (Reich *et al.*, 2003; Lai *et al.*, 2005). Alternatively, the two halves of a large transgene expression cassette contained in dual AAV vectors may contain homologous overlapping sequences (at the 3′ end of the 5′-half vector and at the 5′ end of the 3′-half vector, dual AAV overlapping), which will mediate reconstitution of a single large genome by homologous recombination (Duan *et al.*, 2001). This strategy depends on the recombinogenic properties of the transgene overlapping sequences (Ghosh *et al.*, 2006). A third dual AAV strategy (hybrid) is based on adding a highly recombinogenic region from an exogenous gene (*i.e.*, alkaline phosphatase; Ghosh *et al.*, 2008, Ghosh *et al.*, 2011)) to the trans-splicing vectors. The added region is placed downstream of the SD signal in the 5′-half vector and upstream of the SA signal in the 3′-half vector in order to increase recombination between the dual AAVs.

**[0266]** A “hybrid AAV” or “hybrid rAAV” refers to an rAAV genome packaged with a capsid of a different AAV serotype (and preferably, of a different serotype from the one or more AAV ITRs), and may otherwise be referred to as a pseudotyped rAAV. For example, an rAAV type 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 genome may be encapsidated within an AAV type 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 capsid or variants thereof, provided that the AAV capsid and genome (and preferably, the one or more AAV ITRs) are of different serotypes. In certain embodiments, a pseudotyped rAAV particle may be referred to as being of the type “x/y “, where “x” indicates the source of ITRs and “y” indicates the serotype of capsid, for example a 2/5 rAAV particle has ITRs from AAV2 and a capsid from AAV6.

**[0267]** A “host cell” includes cells transfected, infected, or transduced *in vivo, ex vivo,* or *in vitro* with a recombinant vector or a polynucleotide of the disclosure. Host cells may include virus producing cells and cells infected with viral vectors. In particular embodiments, host cells *in vivo* are infected with viral vector contemplated herein. In certain embodiments, the term “target cell” is used interchangeably with host cell and refers to infected cells of a desired cell type.

**[0268]** High titer AAV preparations can be produced using techniques known in the art, *e.g.*, as described in U.S. Pat. Nos. 5,658,776; 6,566,118; 6,989,264; and 6,995,006; U.S. 2006/0188484; WO98/22607; WO2005/072364; and WO/1999/011764; and Viral Vectors for Gene Therapy: Methods and Protocols, ed. Machida, Humana Press, 2003; Samulski et al., (1989) J. Virology 63, 3822 ; Xiao et al., (1998) J. Virology 72, 2224 ; lnoue et al., (1998) J. Virol. 72, 7024. Methods of producing pseudotyped AAV vectors have also been reported (*e.g.*, WO 00/28004), as well as various modifications or formulations of AAV vectors, to reduce their immunogenicity upon in vivo administration (*see e.g.*, WO 01/23001; WO 00/73316; WO 04/1 12727; WO 05/005610; WO 99/06562).

**G. Pharmaceutical compositions**

**[0269]** In some embodiments, the present disclosure provides compositions comprising a polynucleotide encoding an engineered receptor described herein, or a vector comprising a polynucleotide encoding an engineered receptor described herein. In some embodiments, the compositions further comprise a ligand described here. Pharmaceutical preparations include the subject polynucleotide (RNA or DNA) encoding an engineered receptor, vector carrying a polynucleotide (RNA or DNA) encoding a subject engineered receptor, or ligand present in a pharmaceutically acceptable vehicle. In some embodiments, the present disclosure provides a first composition comprising a polynucleotide encoding an engineered receptor described herein, or a vector comprising a polynucleotide encoding an engineered receptor described herein, and a second composition comprising a ligand described here.

**[0270]** "Pharmaceutically acceptable vehicles" may be vehicles approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in mammals, such as humans. The term "vehicle" refers to a diluent, adjuvant, excipient, or carrier with which a compound of the disclosure is formulated for administration to a mammal. Such pharmaceutical vehicles can be liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. The pharmaceutical vehicles can be saline, gum acacia, gelatin, starch paste, talc, keratin, colloidal silica, urea, and the like. In addition, auxiliary, stabilizing, thickening, lubricating and coloring agents may be used. When administered to a mammal, the compounds and compositions of the disclosure and pharmaceutically acceptable vehicles, excipients, or diluents may be sterile. In some instances, an aqueous medium is employed as a vehicle when the compound of the disclosure is

administered intravenously, such as water, saline solutions, and aqueous dextrose and glycerol solutions.

**[0271]** Pharmaceutical compositions can take the form of capsules, tablets, pills, pellets, lozenges, powders, granules, syrups, elixirs, solutions, suspensions, emulsions, suppositories, or sustained-release formulations thereof, or any other form suitable for administration to a mammal. In some instances, the pharmaceutical compositions are formulated for administration in accordance with routine procedures as a pharmaceutical composition adapted for oral or intravenous administration to humans. Examples of suitable pharmaceutical vehicles and methods for formulation thereof are described in Remington: The Science and Practice of Pharmacy, Alfonso R. Gennaro ed., Mack Publishing Co. Easton, Pa., 19th ed., 1995, Chapters 86, 87, 88, 91, and 92, incorporated herein by reference.

**[0272]** The choice of excipient will be determined in part by the particular vector, as well as by the particular method used to administer the composition. Accordingly, there is a wide variety of suitable formulations of the pharmaceutical composition of the present disclosure.

**[0273]** In some embodiments, the pharmaceutical composition is formulated for subcutaneous administration. In some embodiments, the pharmaceutical composition is formulated for parenteral administration. In some embodiments, the pharmaceutical composition is formulated for intravenous administration. In some embodiments, the pharmaceutical composition is formulated for intramuscular administration. In some embodiments, the pharmaceutical composition is formulated for intradermal administration. In some embodiments, the pharmaceutical composition is formulated for intraperitoneal administration. In some embodiments, the pharmaceutical composition is formulated for oral administration. In some embodiments, the pharmaceutical composition is formulated for infusion. In some embodiments, the pharmaceutical composition is formulated for intracranial administration. In some embodiments, the pharmaceutical composition is formulated for intrathecal administration. In some embodiments, the pharmaceutical composition is formulated for intranasal administration. In some embodiments, the pharmaceutical composition is formulated for intraganglionic administration. In some embodiments, the pharmaceutical composition is formulated for intraspinal administration. In some embodiments, the pharmaceutical composition is formulated for intraventricular administration. In some embodiments, the pharmaceutical composition is formulated for cisterna magna administration. In some embodiments, the pharmaceutical composition is

formulated for intraneural administration. In some embodiments, the pharmaceutical composition is formulated for delivery into a neuronal cell.

**[0274]** For example, the vector may be formulated into preparations for injection by dissolving, suspending or emulsifying them in an aqueous or nonaqueous solvent, such as vegetable or other similar oils, synthetic aliphatic acid glycerides, esters of higher aliphatic acids or propylene glycol; and if desired, with conventional additives such as solubilizers, isotonic agents, suspending agents, emulsifying agents, stabilizers and preservatives.

**[0275]** As another example, the vector may be formulated into a preparation suitable for oral administration, including (a) liquid solutions, such as an effective amount of the compound dissolved in diluents, such as water, or saline; (b) capsules, sachets or tablets, each containing a predetermined amount of the active ingredient, as solids or granules; (c) suspensions in an appropriate liquid; and (d) suitable emulsions. Tablet forms can include one or more of lactose, mannitol, corn starch, potato starch, microcrystalline cellulose, acacia, gelatin, colloidal silicon dioxide, croscarmellose sodium, talc, magnesium stearate, stearic acid, and other excipients, colorants, diluents, buffering agents, moistening agents, preservatives, flavoring agents, and pharmacologically compatible excipients. Lozenge forms can include the active ingredient in a flavor, usually sucrose and acacia or tragacanth, as well as pastilles including the active ingredient in an inert base, such as gelatin and glycerin, or sucrose and acacia, emulsions, gels, and the like containing, in addition to the active ingredient, such excipients as are described herein.

**[0276]** As another example, the subject formulations of the present disclosure can be made into aerosol formulations to be administered via inhalation. These aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like. They may also be formulated as pharmaceuticals for non-pressured preparations such as for use in a nebulizer or an atomizer.

**[0277]** In some embodiments, formulations suitable for parenteral administration include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain anti-oxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. The formulations can be presented in unit-dose or multi-dose sealed containers, such as ampules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid excipient, for example, water, for injections, immediately prior to use.

Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described.

**[0278]** Formulations suitable for topical administration may be presented as creams, gels, pastes, or foams, containing, in addition to the active ingredient, such carriers as are appropriate. In some embodiments the topical formulation contains one or more components selected from a structuring agent, a thickener or gelling agent, and an emollient or lubricant. Frequently employed structuring agents include long chain alcohols, such as stearyl alcohol, and glyceryl ethers or esters and oligo(ethylene oxide) ethers or esters thereof. Thickeners and gelling agents include, for example, polymers of acrylic or methacrylic acid and esters thereof, polyacrylamides, and naturally occurring thickeners such as agar, carrageenan, gelatin, and guar gum. Examples of emollients include triglyceride esters, fatty acid esters and amides, waxes such as beeswax, spermaceti, or carnauba wax, phospholipids such as lecithin, and sterols and fatty acid esters thereof. The topical formulations may further include other components, *e.g.*, astringents, fragrances, pigments, skin penetration enhancing agents, sunscreens (i.e., sunblocking agents), etc.

**[0279]** A compound of the disclosure may be formulated for topical administration. The vehicle for topical application may be in one of various forms, *e.g.* a lotion, cream, gel, ointment, stick, spray, or paste. They may contain various types of carriers, including, but not limited to, solutions, aerosols, emulsions, gels, and liposomes. The carrier may be formulated, for example, as an emulsion, having an oil-in-water or water-in-oil base. Suitable hydrophobic (oily) components employed in emulsions include, for example, vegetable oils, animal fats and oils, synthetic hydrocarbons, and esters and alcohols thereof, including polyesters, as well as organopolysiloxane oils. Such emulsions also include an emulsifier and/or surfactant, *e.g.* a nonionic surfactant to disperse and suspend the discontinuous phase within the continuous phase.

**[0280]** Suppository formulations are also provided by mixing with a variety of bases such as emulsifying bases or water-soluble bases. Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams.

**[0281]** Unit dosage forms for oral or rectal administration such as syrups, elixirs, and suspensions may be provided wherein each dosage unit, for example, teaspoonful, tablespoonful, tablet or suppository, contains a predetermined amount of the composition containing one or more inhibitors. Similarly, unit dosage forms for injection or intravenous administration may include the inhibitor(s) in a composition as a solution in sterile water, normal saline or another pharmaceutically acceptable carrier.

**[0282]** The term "unit dosage form," as used herein, refers to physically discrete units suitable as unitary dosages for human and animal subjects, each unit containing a predetermined quantity of compounds of the present disclosure calculated in an amount sufficient to produce the desired effect in association with a pharmaceutically acceptable diluent, carrier or vehicle. The specifications for the novel unit dosage forms of the present disclosure depend on the particular compound employed and the effect to be achieved, and the pharmacodynamics associated with each compound in the host.

**[0283]** Dose levels can vary as a function of the specific compound, the nature of the delivery vehicle, and the like. Desired dosages for a given compound are readily determinable by a variety of means.

**[0284]** The dose administered to an animal, particularly a human, in the context of the present disclosure should be sufficient to affect a prophylactic or therapeutic response in the animal over a reasonable time frame, *e.g.*, as described in greater detail below. Dosage will depend on a variety of factors including the strength of the particular compound employed, the condition of the animal, and the body weight of the animal, as well as the severity of the illness and the stage of the disease. The size of the dose will also be determined by the existence, nature, and extent of any adverse side-effects that might accompany the administration of a particular compound.

**[0285]** In pharmaceutical dosage forms, the compound(s) may be administered in the form of a free base, their pharmaceutically acceptable salts, or they may also be used alone or in appropriate association, as well as in combination, with other pharmaceutically active compounds.

**H. Clinical Applications and Methods of Treatment**

**[0286]** The compositions and methods disclosed herein can be utilized to treat a neurological disease or disorder. In some aspects of the disclosure, a method of treating a neurological disease or disorder in a subject is provided, the method comprising the introducing an engineered receptor into a neuronal cell and providing a ligand that activates the engineered receptor in an effective amount to control the activity of the cell, thereby relieving pain in the subject. In some aspects, vectors or compositions disclosed herein are used in the manufacture of a medicament for treating a neurological disease or disorder.

**[0287]** In some aspects of the disclosure, a method of treating a neurological disease or disorder in a subject is provided, the method comprises administering an effective amount of a polynucleotide encoding the engineered receptor of the disclosure to a subject. In some

embodiments, the polynucleotide is delivered to a hippocampal neuron. In some embodiments, the polynucleotide is delivered to a dorsal root ganglion neuron or a trigeminal ganglion neuron. In some embodiments, the method further comprises administering a small molecule ligand/drug/agonist that activates the engineered receptor, which in turn modulates the activity of the target neuron, and thereby treating the neurological diseases or disorder (*e.g.*, pain or epilepsy) in the subject.

**[0288]** In some aspects of the disclosure, a method of treating focal epilepsy in a subject is provided, the method comprises administering an effective amount of a polynucleotide encoding the engineered receptor of the disclosure to the subject. In some embodiments, the polynucleotide is delivered into a hippocampal neuron. In some embodiments, the method further comprises administering a small molecule ligand that activates the engineered LGIC, which in turn modulates the activity of the target neuron, and thereby treating the focal epilepsy in the subject.

**[0289]** In some aspects, polynucleotides, vectors, or compositions disclosed herein are used in the manufacture of a medicament for treating a neurological disease or disorder. In some aspects, polynucleotides, vectors, or compositions disclosed herein are used in the manufacture of a medicament for treating a neurological disease or disorder associated with hippocampal neuron dysregulation, such as focal epilepsy, schizophrenia, autism spectrum disorder, Alzheimer's disease, Rett syndrome, and fragile X syndrome. In some aspects, polynucleotides, vectors, or compositions disclosed herein are used for treating focal epilepsy. In some aspects, polynucleotides, vectors, or compositions disclosed herein are used for treating schizophrenia. In some aspects, polynucleotides, vectors, or compositions disclosed herein are used for treating autism spectrum disorder. In some aspects, polynucleotides, vectors, or compositions disclosed herein are used for treating Alzheimer's disease. In some aspects, polynucleotides, vectors, or compositions disclosed herein are used for treating Rett syndrome. In some aspects, polynucleotides, vectors, or compositions disclosed herein are used for treating fragile X syndrome.

**[0290]** The present disclosure contemplates, in part, compositions and methods for controlling, managing, preventing, or treating epilepsy in a subject. In some embodiments, the epilepsy is focal epilepsy. In some embodiments, the focal epilepsy is mesial temporal lobe epilepsy (mTLE).

**[0291]** In some embodiments, the compositions and methods herein may be utilized to ameliorate the level of epileptic seizure in a subject. In some embodiments, the compositions and methods herein may be utilized to prevent or control the level of epileptic seizure in a

subject. Epileptic seizures may be classified as tonic-clonic, tonic, clonic, myoclonic, absence or atonic seizures.

**[0292]** In some embodiments, the compositions and methods herein may reduce the number or frequency of epileptic seizures experienced by a subject by about 5%, about 10%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 99% or 100%, including all ranges and subranges therebetween. In some embodiments, the compositions and methods herein may reduce the number or frequency of epileptic seizures experienced by a subject by at least 5%, at least 10%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99% or 100%, including all ranges and subranges therebetween.

**[0293]** In some embodiments, the compositions and methods herein may reduce the level and/or duration of epileptic seizures experienced by a subject by about 5%, about 10%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 99% or 100%, including all ranges and subranges therebetween. In some embodiments, the compositions and methods herein may reduce the level and/or duration of epileptic seizures experienced by a subject by at least 5%, at least 10%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99% or 100%, including all ranges and subranges therebetween.

**[0294]** In various embodiments, a method for controlling, managing, preventing, or treating epilepsy (e.g., focal epilepsy) in a subject comprises administering to the subject an effective amount of a polynucleotide encoding the engineered receptor of the disclosure. Without wishing to be bound by any particular theory, the present disclosure contemplates using the engineered receptor to modulate neuronal activity to alleviate epilepsy in the subject.

**[0295]** In various embodiments, a vector and/or polynucleotide encoding the engineered receptor that activates or depolarizes neuronal cells is administered to (or introduced into) one or more neuronal cells that controls epilepsy. In the presence of ligand the neuronal cell expressing the engineered receptor, is activated and decreases the sensitivity to epilepsy.

**[0296]** In some embodiments, the epilepsy is focal epilepsy (focal epilepsy seizures). Focal epilepsy is a neurological condition in which the predominant symptom is recurring seizures that affect one hemisphere (half) of the brain.

**[0297]** Focal epilepsy come in four categories: (a) Focal aware seizures, in which the subject is aware of what's happening during the seizure; (b) Focal impaired awareness seizures,; in which the subject is confused or don't know what's happening during the seizure or don't remember it; (c) Focal motor seizures, in which the subject moves to some extent -- anything from twitching, to spasms, to rubbing hands, to walking around; and (d) Focal non-motor seizures, in which the subject does not twitch or make other movements during seizure. Instead, it causes changes in how the subject feels or thinks (e.g., feeling intense emotions, strange feelings, or symptoms like a racing heart, goose bumps, or waves of heat or cold).

**[0298]** Focal epilepsies are characterized by seizures arising from a specific part (lobe) of the brain. Focal epilepsies include idiopathic location-related epilepsies (ILRE), frontal lobe epilepsy, temporal lobe epilepsy, parietal lobe epilepsy and occipital lobe epilepsy.

**[0299]** Idiopathic Localization-Related Epilepsies (ILRE) is caused by unknown factors.

**[0300]** Frontal Lobe Epilepsy is the term for recurring seizures beginning in the frontal lobe — the area of the brain behind the forehead.

**[0301]** Temporal lobe epilepsy is the term for recurring seizures beginning in the temporal lobe – the section of the brain located on the sides of the head behind the temples and cheekbones. The temporal lobes are the areas of the brain that most commonly give rise to seizures. The mesial portion (middle) of both temporal lobes is very important in epilepsy — it is frequently the source of seizures and can be prone to damage or scarring. Mesial temporal lobe epilepsy (mTLE) is the most common form of human epilepsy. Often times, its pathophysiological substrate is hippocampal sclerosis. Thus, one strategy of treating focal epilepsy such as mTLE is by targeting hippocampal neurons.

**[0302]** Parietal Lobe Epilepsy. The parietal lobe is the section of the brain on the top and sides of the head. Known as the “association cortex,” the parietal lobe is responsible for connecting meaning to the brain’s functions. It is here that the brain creates a visual image, that sounds are recognized as words, and that the sense of touch is associated with a particular object. In some ways, the parietal lobe is where perception meshes with physical reality.

**[0303]** Occipital Lobe Epilepsy. Occipital lobe epilepsy is the term for recurring seizures beginning in the occipital lobe, the section of the brain in the back of the head that is primarily responsible for vision.

**[0304]** In some aspects of the disclosure, a method of treating neuropathic pain, such as Peripheral Neuropathy and Trigeminal Neuralgia, in a subject is provided, the method comprises administering an effective amount of a polynucleotide to the subject, wherein the polynucleotide encodes an engineered receptor of the disclosure. In some embodiments, the polynucleotide is delivered into a DRG or TGG neuron. In some embodiments, the method further comprises administering a small molecule ligand that activates the engineered LGIC, which in turn modulates the activity of the target neuron, and thereby treating neuropathic pain, such as Peripheral Neuropathy and Trigeminal Neuralgia, in the subject.

**[0305]** In some aspects, polynucleotides, vectors, or compositions disclosed herein are used in the manufacture of a medicament for treating a neurological disease or disorder. In some aspects, polynucleotides, vectors, or compositions disclosed herein are used in the manufacture of a medicament for treating a neurological disease or disorder associated with DRG/TGG neuron dysregulation, such as neuropathic pain. In some aspects, polynucleotides, vectors, or compositions disclosed herein are used in the manufacture of a medicament for treating a spinal cord related disease such as spasticity, spinal cord injury, and avulsion injury. In some aspects, polynucleotides, vectors, or compositions disclosed herein are used for treating spasticity. In some embodiments, treating spasticity comprises transducing neurons in the spinal column. In some aspects, polynucleotides, vectors, or compositions disclosed herein are used for treating spinal cord injury. In some aspects, polynucleotides, vectors, or compositions disclosed herein are used for treating avulsion injury. In some embodiments, treating spinal cord injury or avulsion injury comprises transducing a neuron in the dorsal horn.

**[0306]** In some embodiments, the neuropathic pain is peripheral neuropathy. Peripheral neuropathy refers to the conditions that result when nerves that carry messages to and from the brain and spinal cord from and to the rest of the body are damaged or diseased. Various kinds of peripheral neuropathy range from carpal tunnel syndrome (a traumatic injury common after chronic repetitive use of the hands and wrists, such as with computer use) to nerve damage linked to diabetes. In general, peripheral neuropathy can be categorized into mononeuropathy and polyneuropathy. Mononeuropathy includes carpal tunnel syndrome, ulnar nerve palsy, radial nerve palsy, and peroneal nerve palsy. Polyneuropathy occurs when multiple peripheral nerves throughout the body malfunction at the same time. Polyneuropathy can have a wide variety of causes, including exposure to certain toxins such as with alcohol abuse, poor nutrition (particularly vitamin B deficiency), and complications from diseases such as cancer or kidney failure. One of the most common forms of chronic polyneuropathy is diabetic neuropathy, a condition that occurs in people with diabetes. It is more severe in people with

poorly controlled blood sugar levels. Though less common, diabetes can also cause a mononeuropathy. One of the most serious polyneuropathies is Guillain-Barre syndrome, a rare disease that strikes suddenly when the body's immune system attacks nerves in the body just as they leave the spinal cord. Symptoms tend to appear quickly and worsen rapidly, sometimes leading to paralysis. Early symptoms include weakness and tingling that eventually may spread upward into the arms. Blood pressure problems, heart rhythm problems, and breathing difficulty may occur in the more severe cases. Chronic inflammatory demyelinating polyneuropathy (CIDP) is a chronic form of Guillain-Barre in which the symptoms continue for months and even years. Early diagnosis and treatment is crucial for CIDP patients, 30% of which risk eventually being confined to a wheelchair.

**[0307]** In some embodiments, the neuropathic pain is trigeminal neuralgia. Trigeminal neuralgia (TN), also called tic douloureux, is a chronic pain condition that affects the trigeminal or 5th cranial nerve, one of the most widely distributed nerves in the head. The trigeminal nerve is one set of the cranial nerves in the head. It is the nerve responsible for providing sensation to the face. One trigeminal nerve runs to the right side of the head, while the other runs to the left. Each of these nerves has three distinct branches. After the trigeminal nerve leaves the brain and travels inside the skull, it divides into three smaller branches, controlling sensations throughout the face: Ophthalmic Nerve (V1): The first branch controls sensation in a person's eye, upper eyelid and forehead. Maxillary Nerve (V2): The second branch controls sensation in the lower eyelid, cheek, nostril, upper lip and upper gum. Mandibular Nerve (V3): The third branch controls sensations in the jaw, lower lip, lower gum and some of the muscles used for chewing.

**[0308]** TN is a form of neuropathic pain. The typical or "classic" form of the disorder (called "Type 1" or TN1) causes extreme, sporadic, sudden burning or shock-like facial pain that lasts anywhere from a few seconds to as long as two minutes per episode. These attacks can occur in quick succession, in volleys lasting as long as two hours. The “atypical” form of the disorder (called "Type 2" or TN2), is characterized by constant aching, burning, stabbing pain of somewhat lower intensity than Type 1. Both forms of pain may occur in the same person, sometimes at the same time. The intensity of pain can be physically and mentally incapacitating. TN is associated with a variety of conditions. TN can be caused by a blood vessel pressing on the trigeminal nerve as it exits the brain stem. This compression causes the wearing away or damage to the protective coating around the nerve (the myelin sheath). TN symptoms can also occur in people with multiple sclerosis, a disease that causes deterioration of the trigeminal nerve’s myelin sheath. Rarely, symptoms of TN may be caused by nerve

compression from a tumor, or a tangle of arteries and veins called an arteriovenous malformation. Injury to the trigeminal nerve (perhaps the result of sinus surgery, oral surgery, stroke, or facial trauma) may also produce neuropathic facial pain..

**[0309]** In some cases, the methods and compositions of the disclosure are utilized to treat epilepsy. Compositions described herein may be used to prevent or control epileptic seizures. Epileptic seizures may be classified as tonic-clonic, tonic, clonic, myoclonic, absence or atonic seizures. In some cases, the compositions and methods herein may prevent or reduce the number of epileptic seizures experienced by a subject by about 5%, about 10%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 99% or 100%, including all ranges and subranges therebetween.

**[0310]** In some cases, the methods and compositions of the disclosure are utilized to treat an eating disorder. An eating disorder may be a mental disorder defined by abnormal eating behaviors that negatively affect a subject's physical or mental health. In some cases, the eating disorder is anorexia nervosa. In other cases, the eating disorder is bulimia nervosa. In some cases, the eating disorder is pica, rumination disorder, avoidant/restrictive food intake disorder, binge eating disorder (BED), other specified feeding and eating disorder (OSFED), compulsive overeating, diabulimia, orthorexia nervosa, selective eating disorder, drunkorexia, pregorexia, or Gourmand syndrome. In some cases, the composition includes a G-protein coupled receptor that increases or decreases the production of one or more molecules associated with an eating disorder. In other cases, the composition includes a ligand-gated ion channel that alters the production of one or more molecules associated with an eating disorder. The one or more molecules associated with an eating disorder may include, without limitation, a molecule of the hypothalamus-pituitary-adrenal (HPA) axis, including vasopressin, corticotropin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH), cortisol, epinephrine, or norepinephrine; as well as serotonin, dopamine, neuropeptide Y, leptin, or ghrelin.

**[0311]** In some cases, the compositions and methods are utilized to treat post-traumatic stress disorder (PTSD), gastroesophageal reflex disease (GERD), addiction (*e.g.*, alcohol, drugs), anxiety, depression, memory loss, dementia, sleep apnea, stroke, urinary incontinence, narcolepsy, essential tremor, movement disorder, atrial fibrillation, cancer (*e.g.*, brain tumors), Parkinson's disease, or Alzheimer's disease. Other non-limiting examples of neurological diseases or disorders that can be treated by the compositions and methods herein include: Abulia, Agraphia, Alcoholism, Alexia, Aneurysm, Amaurosis fugax, Amnesia, Amyotrophic

lateral sclerosis (ALS), Angelman syndrome, Aphasia, Apraxia, Arachnoiditis, Arnold-Chiari malformation, Asperger syndrome, Ataxia, Ataxia-telangiectasia, Attention deficit hyperactivity disorder, Auditory processing disorder, Autism spectrum, Bipolar disorder, Bell's palsy, Brachial plexus injury, Brain damage, Brain injury, Brain tumor, Canavan disease, Capgras delusion, Carpal tunnel syndrome, Causalgia, Central pain syndrome, Central pontine myelinolysis, Centronuclear myopathy, Cephalic disorder, Cerebral aneurysm, Cerebral arteriosclerosis, Cerebral atrophy, Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), Cerebral gigantism, Cerebral palsy, Cerebral vasculitis, Cervical spinal stenosis, Charcot-Marie-Tooth disease, Chiari malformation, Chorea, Chronic fatigue syndrome, Chronic inflammatory demyelinating polyneuropathy (CIDP), Chronic pain, Coffin–Lowry syndrome, Coma, Complex regional pain syndrome, Compression neuropathy, Congenital facial diplegia, Corticobasal degeneration, Cranial arteritis, Craniosynostosis, Creutzfeldt-Jakob disease, Cumulative trauma disorders, Cushing's syndrome, Cyclothymic disorder, Cytomegalic inclusion body disease (CIBD), Cytomegalovirus Infection, Dandy-Walker syndrome, Dawson disease, De Morsier's syndrome, Dejerine-Klumpke palsy, Dejerine-Sottas disease, Delayed sleep phase syndrome, Dementia, Dermatomyositis, Developmental coordination disorder, Diabetic neuropathy, Diffuse sclerosis, Diplopia, Down syndrome, Dravet syndrome, Duchenne muscular dystrophy, Dysarthria, Dysautonomia, Dyscalculia, Dysgraphia, Dyskinesia, Dyslexia, Dystonia, Empty sella syndrome, Encephalitis, Encephalocele, Encephalotrigeminal angiomatosis, Encopresis, Enuresis, Epilepsy, Epilepsy-intellectual disability in females, Erb's palsy, Erythromelalgia, Exploding head syndrome, Fabry's disease, Fahr's syndrome, Fainting, Familial spastic paralysis, Febrile seizures, Fisher syndrome, Friedreich's ataxia, Fibromyalgia, Foville's syndrome, Fetal alcohol syndrome, Fragile X syndrome, Fragile X-associated tremor/ataxia syndrome (FXTAS), Gaucher's disease, Generalized epilepsy with febrile seizures plus, Gerstmann's syndrome, Giant cell arteritis, Giant cell inclusion disease, Globoid Cell Leukodystrophy, Gray matter heterotopia, Guillain-Barré syndrome, Generalized anxiety disorder, HTLV-1 associated myelopathy, Hallervorden-Spatz disease, Head injury, Headache, Hemifacial Spasm, Hereditary Spastic Paraplegia, Heredopathia atactica polyneuritiformis, Herpes zoster oticus, Herpes zoster, Hirayama syndrome, Hirschsprung's disease, Holmes-Adie syndrome, Holoprosencephaly, Huntington's disease, Hydranencephaly, Hydrocephalus, Hypercortisolism, Hypoxia, Immune-Mediated encephalomyelitis, Inclusion body myositis, Incontinentia pigmenti, Infantile Refsum disease, Infantile spasms, Inflammatory myopathy, Intracranial cyst, Intracranial hypertension, Isodicentric 15, Joubert

syndrome, Karak syndrome, Kearns-Sayre syndrome, Kinsbourne syndrome, Kleine-Levin Syndrome, Klippel Feil syndrome, Krabbe disease, Lafora disease, Lambert-Eaton myasthenic syndrome, Landau-Kleffner syndrome, Lateral medullary (Wallenberg) syndrome, Learning disabilities, Leigh's disease, Lennox-Gastaut syndrome, Lesch-Nyhan syndrome, Leukodystrophy, Leukoencephalopathy with vanishing white matter, Lewy body dementia, Lissencephaly, Locked-In syndrome, Lumbar disc disease, Lumbar spinal stenosis, Lyme disease - Neurological Sequelae, Machado-Joseph disease (Spinocerebellar ataxia type 3), Macrencephaly, Macropsia, Mal de debarquement, Megalencephalic leukoencephalopathy with subcortical cysts, Megalencephaly, Melkersson-Rosenthal syndrome, Menieres disease, Meningitis, Menkes disease, Metachromatic leukodystrophy, Microcephaly, Micropsia, Migraine, Miller Fisher syndrome, Mini-stroke (transient ischemic attack), Misophonia, Mitochondrial myopathy, Mobius syndrome, Monomelic amyotrophy, Motor skills disorder, Moyamoya disease, Mucopolysaccharidoses, Multi-infarct dementia, Multifocal motor neuropathy, Multiple sclerosis, Multiple system atrophy, Muscular dystrophy, Myalgic encephalomyelitis, Myasthenia gravis, Myelinoclastic diffuse sclerosis, Myoclonic Encephalopathy of infants, Myoclonus, Myopathy, Myotubular myopathy, Myotonia congenita, Narcolepsy, Neuro-Behçet's disease, Neurofibromatosis, Neuroleptic malignant syndrome, Neurological manifestations of AIDS, Neurological sequelae of lupus, Neuromyotonia, Neuronal ceroid lipofuscinosis, Neuronal migration disorders, Neuropathy, Neurosis, Niemann-Pick disease, Non-24-hour sleep–wake disorder, Nonverbal learning disorder, O'Sullivan-McLeod syndrome, Occipital Neuralgia, Occult Spinal Dysraphism Sequence, Ohtahara syndrome, Olivopontocerebellar atrophy, Opsoclonus myoclonus syndrome, Optic neuritis, Orthostatic Hypotension, Otosclerosis, Overuse syndrome, Palinopsia, Paresthesia, Parkinson's disease, Paramyotonia Congenita, Paraneoplastic diseases, Paroxysmal attacks, Parry-Romberg syndrome, PANDAS, Pelizaeus-Merzbacher disease, Periodic Paralyses, Peripheral neuropathy, Pervasive developmental disorders, Photic sneeze reflex, Phytanic acid storage disease, Pick's disease, Pinched nerve, Pituitary tumors, PMG, Polyneuropathy, Polio, Polymicrogyria, Polymyositis, Porencephaly, Post-Polio syndrome, Postherpetic Neuralgia (PHN), Postural Hypotension, Prader-Willi syndrome, Primary Lateral Sclerosis, Prion diseases, Progressive hemifacial atrophy, Progressive multifocal leukoencephalopathy, Progressive Supranuclear Palsy, Prosopagnosia, Pseudotumor cerebri, Quadrantanopia, Quadriplegia, Rabies, Radiculopathy, Ramsay Hunt syndrome type I, Ramsay Hunt syndrome type II, Ramsay Hunt syndrome type III, Rasmussen encephalitis, Reflex neurovascular dystrophy, Refsum disease, REM sleep behavior disorder,

Repetitive stress injury, Restless legs syndrome, Retrovirus-associated myelopathy, Rett syndrome, Reye's syndrome, Rhythmic Movement Disorder, Romberg syndrome, Saint Vitus dance, Sandhoff disease, Schilder's disease, Schizencephaly, Sensory processing disorder, Septo-optic dysplasia, Shaken baby syndrome, Shingles, Shy-Drager syndrome, Sjögren's syndrome, Sleep apnea, Sleeping sickness, Snatiation, Sotos syndrome, Spasticity, Spina bifida, Spinal cord injury, Spinal cord tumors, Spinal muscular atrophy, Spinal and bulbar muscular atrophy, Spinocerebellar ataxia, Split-brain, Steele-Richardson-Olszewski syndrome, Stiff-person syndrome, Stroke, Sturge-Weber syndrome, Stuttering, Subacute sclerosing panencephalitis, Subcortical arteriosclerotic encephalopathy, Superficial siderosis, Sydenham's chorea, Syncope, Synesthesia, Syringomyelia, Tarsal tunnel syndrome, Tardive dyskinesia, Tardive dysphrenia, Tarlov cyst, Tay-Sachs disease, Temporal arteritis, Temporal lobe epilepsy, Tetanus, Tethered spinal cord syndrome, Thomsen disease, Thoracic outlet syndrome, Tic Douloureux, Todd's paralysis, Tourette syndrome, Toxic encephalopathy, Transient ischemic attack, Transmissible spongiform encephalopathies, Transverse myelitis, Traumatic brain injury, Tremor, Trichotillomania, Trigeminal neuralgia, Tropical spastic paraparesis, Trypanosomiasis, Tuberous sclerosis, Unverricht-Lundborg disease, Von Hippel-Lindau disease (VHL), Viliuisk Encephalomyelitis (VE), Wallenberg's syndrome, West syndrome, Whiplash, Williams syndrome, Wilson's disease, or Zellweger syndrome.

**[0312]** In some cases, the compositions and methods disclosed herein can be used to treat brain cancer or brain tumors. Non-limiting examples of brain cancers or tumors that may be amenable to treatment with vectors and compositions described herein include: gliomas including anaplastic astrocytoma (grade III glioma), astrocytoma (grade II glioma), brainstem glioma, ependymoma, ganglioglioma, ganglioneuroma, glioblastoma (grade IV glioma), glioma, juvenile pilocytic astrocytoma (JPA), low-grade astrocytoma (LGA), medullablastoma, mixed glioma, oligodendroglioma, optic nerve glioma, pilocytic astrocytoma (grade I glioma), and primitive neuroectodermal (PNET); skull base tumors including acoustic neuroma (vestibular schwannoma), acromegaly, adenoma, chondrosarcoma, chordoma, craniopharyngioma, epidermoid tumor, glomus jugulare tumor, infratentorial meningioma, meningioma, pituitary adenoma, pituitary tumor, Rathke's cleft cyst; metastatic cancer including brain metastasis, metastatic brain tumor; other brain tumors including brain cyst, choroid plexus papilloma, CNS lymphoma, colloid cyst, cystic tumor, dermoid tumor, germinoma, lymphoma, nasal carcinoma, naso-pharyngeal tumor, pineal tumor, pineoblastoma, pineocytoma, supratentorial meningioma, and vascular tumor; spinal cord tumors including astrocytoma, ependymoma, meningioma, and schwannoma.

**[0313]** The present disclosure contemplates, in part, compositions and methods for controlling, managing, preventing, or treating pain in a subject. "Pain" refers to an uncomfortable feeling and/or an unpleasant sensation in the body of a subject. Feelings of pain can range from mild and occasional to severe and constant. Pain can be classified as acute pain or chronic pain. Pain can be nociceptive pain (i.e., pain caused by tissue damage), neuropathic pain or psychogenic pain. In some cases, the pain is caused by or associated with a disease (*e.g.*, cancer, arthritis, diabetes). In other cases, the pain is caused by injury (*e.g.*, sports injury, trauma). Non-limiting examples of pain that are amenable to treatment with the compositions and methods herein include: neuropathic pain including peripheral neuropathy, diabetic neuropathy, post herpetic neuralgia, trigeminal neuralgia, back pain, neuropathy associated with cancer, neuropathy associated with HIV/AIDS, phantom limb pain, carpal tunnel syndrome, central post-stroke pain, pain associated with chronic alcoholism, hypothyroidism, uremia, pain associated with multiple sclerosis, pain associated with spinal cord injury, pain associated with Parkinson's disease, epilepsy, osteoarthritic pain, rheumatoid arthritic pain, visceral pain, and pain associated with vitamin deficiency; and nociceptive pain including pain associated with central nervous system trauma, strains/sprains, and burns; myocardial infarction, acute pancreatitis, post-operative pain, posttraumatic pain, renal colic, pain associated with cancer, pain associated with fibromyalgia, pain associated with carpal tunnel syndrome, and back pain.

**[0314]** The compositions and methods herein may be utilized to ameliorate a level of pain in a subject. In some cases, a level of pain in a subject is ameliorated by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least 99% or about 100%, including all ranges and subranges therebetween. A level of pain in a subject can be assessed by a variety of methods. In some cases, a level of pain is assessed by self-reporting (i.e., a human subject expresses a verbal report of the level of pain he/she is experiencing). In some cases, a level of pain is assessed by behavioral indicators of pain, for example, facial expressions, limb movements, vocalization, restlessness and guarding. These types of assessments may be useful for example when a subject is unable to self-report (*e.g.*, an infant, an unconscious subject, a non-human subject). A level of pain may be assessed after treatment with a composition of the disclosure as compared to the level of pain the subject was experiencing prior to treatment with the composition.

**[0315]** In various embodiments, a method for controlling, managing, preventing, or treating pain in a subject comprises administering to the subject an effective amount of an engineered receptor contemplated herein. Without wishing to be bound by any particular theory, the present disclosure contemplates using the vectors disclosed herein to modulate neuronal activity to alleviate pain in the subject.

**[0316]** In various embodiments, a vector encoding an engineered receptor that activates or depolarizes neuronal cells is administered to (or introduced into) one or more neuronal cells that decrease pain sensation, *e.g.,* inhibitory interneurons. In the presence of ligand, the neuronal cell expressing the engineered receptor, is activated and decreases the sensitivity to pain potentiating the analgesic effect of stimulating these neuronal cells.

**[0317]** In various embodiments, a vector encoding an engineered receptor that deactivates or hyperpolarizes neuronal cells is administered to (or introduced into) one or more neuronal cells that increase pain sensation or sensitivity to pain, *e.g.,* nociceptor, peripheral sensory neurons, C-fibers, Aδ fibers, Aβ fibers, DRG neurons, TGG neurons, and the like. In the presence of ligand, the neuronal cell expressing the engineered receptor, is deactivated and decreases the sensitivity to pain and potentiating an analgesic effect.

**[0318]** Targeting expression of an engineered receptor to a sub-population of nociceptors can be achieved by one or more of: selection of the vector (*e.g.*, AAV1, AAV1(Y705+731F+T492V), AAV2(Y444+500+730F+T491V), AAV3(Y705+731F), AAV5, AAV5(Y436+693+719F), AAV6, AAV6 (VP3 variant Y705F/Y731F/T492V), AAV-7m8, AAV8, AAV8(Y733F), AAV9, AAV9 (VP3 variant Y731F), AAV10(Y733F), and AAV-ShH10); selection of a promoter; and delivery means.

**[0319]** In particular embodiments, the compositions and methods contemplated herein are effective in reducing pain. Illustrative examples of pain that are amenable to treatment with the vectors, compositions, and methods contemplated herein, include but are not limited to acute pain, chronic pain, neuropathic pain, nociceptive pain, allodynia, inflammatory pain, inflammatory hyperalgesia, neuropathies, neuralgia, diabetic neuropathy, human immunodeficiency virus-related neuropathy, nerve injury, rheumatoid arthritic pain, osteoarthritic pain, burns, back pain, eye pain, visceral pain, cancer pain (*e.g.*, bone cancer pain), dental pain, headache, migraine, carpal tunnel syndrome, fibromyalgia, neuritis, sciatica, pelvic hypersensitivity, pelvic pain, post herpetic neuralgia, post-operative pain, post stroke pain, and menstrual pain.

**[0320]** Pain can be classified as acute or chronic. "Acute pain" refers to pain that begins suddenly and is usually sharp in quality. Acute pain might be mild and last just a moment, or it might be severe and last for weeks or months. In most cases, acute pain does not last longer than three months, and it disappears when the underlying cause of pain has been treated or has healed. Unrelieved acute pain, however, may lead to chronic pain. "Chronic pain" refers to ongoing or recurrent pain, lasting beyond the usual course of acute illness or injury or lasting for more than three to six months, and which adversely affects the individual's well-being. In particular embodiments, the term "chronic pain" refers to pain that continues when it should not. Chronic pain can be nociceptive pain or neuropathic pain.

**[0321]** In some embodiments, the pain is expected or anticipated to develop in association with or as a result of an injury, an infection, or a medical intervention. In some embodiments, the infection causes nerve damage. In some embodiments, the medical intervention is a surgery, such as surgery to the central core of the body. In some embodiments, the medical intervention is a surgery to remove parts or whole of one or more tissues, tumors or organs in the body. In some embodiments, the medical intervention is an amputation. In particular embodiments, the compositions and methods contemplated herein are effective in reducing acute pain. In particular embodiments, the compositions and methods contemplated herein are effective in reducing chronic pain.

**[0322]** Clinical pain is present when discomfort and abnormal sensitivity feature among the patient's symptoms. Individuals can present with various pain symptoms. Such symptoms include: 1) spontaneous pain which may be dull, burning, or stabbing; 2) exaggerated pain responses to noxious stimuli (hyperalgesia); and 3) pain produced by normally innocuous stimuli (allodynia-Meyer et al., 1994, Textbook of Pain, 13-44). Although patients suffering from various forms of acute and chronic pain may have similar symptoms, the underlying mechanisms may be different and may, therefore, require different treatment strategies. Pain can also therefore be divided into a number of different subtypes according to differing pathophysiology, including nociceptive pain, inflammatory pain, and neuropathic pain.

**[0323]** In particular embodiments, the compositions and methods contemplated herein are effective in reducing nociceptive pain. In particular embodiments, the compositions and methods contemplated herein are effective in reducing inflammatory pain. In particular embodiments, the compositions and methods contemplated herein are effective in reducing neuropathic pain.

**[0324]** Nociceptive pain is induced by tissue injury or by intense stimuli with the potential to cause injury. Moderate to severe acute nociceptive pain is a prominent feature of pain from central nervous system trauma, strains/sprains, burns, myocardial infarction and acute pancreatitis, post-operative pain (pain following any type of surgical procedure), posttraumatic pain, renal colic, cancer pain and back pain. Cancer pain may be chronic pain such as tumor related pain (*e.g.*, bone pain, headache, facial pain or visceral pain) or pain associated with cancer therapy (*e.g.*, post chemotherapy syndrome, chronic postsurgical pain syndrome or post radiation syndrome). Cancer pain may also occur in response to chemotherapy, immunotherapy, hormonal therapy or radiotherapy. Back pain may be due to herniated or ruptured intervertebral discs or abnormalities of the lumber facet joints, sacroiliac joints, paraspinal muscles or the posterior longitudinal ligament. Back pain may resolve naturally but in some patients, where it lasts over 12 weeks, it becomes a chronic condition which can be particularly debilitating.

**[0325]** Neuropathic pain can be defined as pain initiated or caused by a primary lesion or dysfunction in the nervous system. Etiologies of neuropathic pain include, *e.g.*, peripheral neuropathy, diabetic neuropathy, post herpetic neuralgia, trigeminal neuralgia, back pain, cancer neuropathy, HIV neuropathy, phantom limb pain, carpal tunnel syndrome, central post-stroke pain and pain associated with chronic alcoholism, hypothyroidism, uremia, multiple sclerosis, spinal cord injury, Parkinson's disease, epilepsy, and vitamin deficiency.

**[0326]** Neuropathic pain can be related to a pain disorder, a term referring to a disease, disorder or condition associated with or caused by pain. Illustrative examples of pain disorders include arthritis, allodynia, a typical trigeminal neuralgia, trigeminal neuralgia, somatoform disorder, hypoesthesis, hypealgesia, neuralgia, neuritis, neurogenic pain, analgesia, anesthesia dolorosa, causlagia, sciatic nerve pain disorder, degenerative joint disorder, fibromyalgia, visceral disease, chronic pain disorders, migraine/headache pain, chronic fatigue syndrome, complex regional pain syndrome, neurodystrophy, plantar fasciitis or pain associated with cancer.

**[0327]** The inflammatory process is a complex series of biochemical and cellular events, activated in response to tissue injury or the presence of foreign substances, which results in swelling and pain. Arthritic pain is a common inflammatory pain.

**[0328]** Other types of pain that are amenable to treatment with the vectors, compositions, and methods contemplated herein, include but are not limited to pain resulting from musculoskeletal disorders, including myalgia, fibromyalgia, spondylitis, sero-negative (non-rheumatoid) arthropathies, non-articular rheumatism, dystrophinopathy, glycogenolysis,

polymyositis and pyomyositis; heart and vascular pain, including pain caused by angina, myocardical infarction, mitral stenosis, pericarditis, Raynaud's phenomenon, scleredoma and skeletal muscle ischemia; head pain, such as migraine (including migraine with aura and migraine without aura), cluster headache, tension-type headache mixed headache and headache associated with vascular disorders; and orofacial pain, including dental pain, otic pain, burning mouth syndrome, and temporomandibular myofascial pain.

**[0329]** The effective amount of the compositions and methods contemplated herein to reduce the amount of pain experienced by a human subject can be determined using a variety of pain scales. Patient self-reporting can be used to assess whether pain is reduced; see, *e.g.*, Katz and Melzack (1999) Surg. Clin. North Am. 79:231. Alternatively, an observational pain scale can be used. The LANSS Pain Scale can be used to assess whether pain is reduced; see, *e.g.*, Bennett (2001) Pain 92:147. A visual analog pain scale can be used; see, *e.g.*, Schmader (2002) Clin. J. Pain 18:350. The Likert pain scale can be used; *e.g.*, where 0 is no pain, 5 is moderate pain, and 10 is the worst pain possible. Self -report pain scales for children include, *e.g.*, Faces Pain Scale; Wong-Baker FACES Pain Rating Scale; and Colored Analog Scale. Self-report pain scales for adults include, *e.g.*, Visual Analog Scale; Verbal Numerical Rating Scale; Verbal Descriptor Scale; and Brief Pain Inventory. Pain measurement scales include, *e.g.*, Alder Hey Triage Pain Score (Stewart et al. (2004) Arch. Dis. Child. 89:625); Behavioral Pain Scale (Payen et al. (2001) Critical Care Medicine 29:2258); Brief Pain Inventory (Cleeland and Ryan (1994) Ann. Acad. Med. Singapore 23: 129); Checklist of Nonverbal Pain Indicators (Feldt (2000) Pain Manag. Nurs. 1 : 13); Critical-Care Pain Observation Tool (Gelinas et al. (2006) Am. J. Crit. Care 15:420); COMFORT scale (Ambuel et al. (1992) J. Pediatric Psychol. 17:95); Dallas Pain Questionnaire (Ozguler et al. (2002) Spine 27:1783); Dolorimeter Pain Index (Hardy et al. (1952) Pain Sensations and Reactions Baltimore: The Williams & Wilkins Co.); Faces Pain Scale - Revised (Hicks et al. (2001) Pain 93:173); Face Legs Activity Cry Consolability Scale; McGill Pain Questionnaire (Melzack (1975) Pain 1 :277); Descriptor Differential Scale (Gracely and Kwilosz (1988) Pain 35:279); Numerical 1 1 point Box (Jensen et al. (1989) Clin. J. Pain 5: 153); Numeric Rating Scale (Hartrick et al. (2003) Pain Pract. 3:310); Wong-Baker FACES Pain Rating Scale; and Visual Analog Scale (Huskisson (1982) J. Rheumatol. 9:768).

**[0330]** In particular embodiments, a method of relieving pain in a subject is provided, the method comprising introducing an engineered receptor into a neuronal cell and controlling the activity of the cell by providing an effective amount of a ligand that activates the engineered receptor, thereby relieving pain in the subject. The method provides significant analgesia for

pain without off-target effects, such as general central nervous system depression. In certain embodiments, the method provides a 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more including all ranges and subranges therebetween) reduction in the neuropathic pain in a subject compared to an untreated subject. In some embodiments, the method comprises the step of measuring pain in the subject before and after the administration of the ligand, wherein the pain in the subject is reduced 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more, including all ranges and subranges therebetween. In such instances, the measuring may occur 4 hours or more after administration of the ligand, e.g. 8 hours 12 hours, 16 hours, 24 hours, 36 hours, 48 hours, 3 days, or 4 days or more after administration of the ligand.

**[0331]** In particular embodiments, the vectors contemplated herein are administered or introduced into one or more neuronal cells. The neuronal cells may be the same type of neuronal cells, or a mixed population of different types of neuronal cells. In one embodiment, the neuronal cell is a nociceptor or peripheral sensory neuron. Illustrative examples of sensory neurons include, but are not limited to, dorsal root ganglion (DRG) neurons and trigeminal ganglion (TGG) neurons. In one embodiment, the neuronal cell is an inhibitory interneuron involved in the neuronal pain circuit.

**[0332]** In some cases, a vector encoding an engineered receptor is administered to a subject in need thereof. Non-limiting examples of methods of administration include subcutaneous administration, intravenous administration, intramuscular administration, intradermal administration, intraperitoneal administration, oral administration, infusion, intracranial administration, intrathecal administration, intranasal administration, intraganglionic administration, intraspinal administration, cisterna magna administration and intraneural administration. In some cases, administration can involve injection of a liquid formulation of the vector. In other cases, administration can involve oral delivery of a solid formulation of the vector. In some cases, the oral formulation can be administered with food. In particular embodiments, a vector is parenterally, intravenously, intramuscularly, intraperitoneally, intrathecally, intraneurally, intraganglionicly, intraspinally, or intraventricularly administered to a subject in order to introduce the vector into one or more neuronal cells. In various embodiments, the vector is rAAV.

**[0333]** In one embodiment, AAV is administered to sensory neuron or nociceptor, *e.g.*, DRG neurons, TGG neurons, etc. by intrathecal (IT) or intraganglionic (IG) administration.

The IT route delivers AAV to the cerebrospinal fluid (CSF). This route of administration may be suitable for the treatment of *e.g.*, chronic pain or other peripheral nervous system (PNS) or central nervous system (CNS) indications. In animals, IT administration has been achieved by inserting an IT catheter through the *cisterna magna* and advancing it caudally to the lumbar level. In humans, IT delivery can be easily performed by lumbar puncture (LP), a routine bedside procedure with excellent safety profile.

**[0334]** In a particular case, a vector may be administered to a subject by intraganglionic administration. Intraganglionic administration may involve an injection directly into one or more ganglia. The IG route may deliver AAV directly into the DRG or TGG parenchyma. In animals, IG administration to the DRG is performed by an open neurosurgical procedure that is not desirable in humans because it would require a complicated and invasive procedure. In humans, a minimally invasive, CT imaging-guided technique to safely target the DRG can be used. A customized needle assembly for convection enhanced delivery (CED) can be used to deliver AAV into the DRG parenchyma. In a non-limiting example, a vector of the disclosure may be delivered to one or more dorsal root ganglia and/or trigeminal ganglia for the treatment of chronic pain. In another non-limiting example, a vector of the disclosure may be delivered to the nodose ganglion (vagus nerve) to treat epilepsy.

**[0335]** In yet another particular case, a vector may be administered to the subject by intracranial administration (i.e., directly into the brain). In non-limiting examples of intracranial administration, a vector of the disclosure may be delivered into the cortex of the brain to treat *e.g.*, an epileptic seizure focus, into the paraventricular hypothalamus to treat *e.g.*, a satiety disorder, or into the amygdala central nucleus to treat *e.g.*, a satiety disorder. In another particular case, a vector may be administered to a subject by intraneural injection (i.e., directly into a nerve). The nerve may be selected based on the indication to be treated, for example, injection into the sciatic nerve to treat chronic pain or injection into the vagal nerve to treat epilepsy or a satiety disorder. In yet another particular case, a vector may be administered to a subject by subcutaneous injection, for example, into the sensory nerve terminals to treat chronic pain.

**[0336]** A vector dose may be expressed as the number of vector genome units delivered to a subject. A "vector genome unit" as used herein refers to the number of individual vector genomes administered in a dose. The size of an individual vector genome will generally depend on the type of viral vector used. Vector genomes of the disclosure may be from about 1.0 kilobase, 1.5 kilobases, 2.0 kilobases, 2.5 kilobases, 3.0 kilobases, 3.5 kilobases, 4.0 kilobases, 4.5 kilobases, 5.0 kilobases, 5.5 kilobases, 6.0 kilobases, 6.5 kilobases, 7.0 kilobases, 7.5

kilobases, 8.0 kilobases, 8.5 kilobases, 9.0 kilobases, 9.5 kilobases, 10.0 kilobases, to more than 10.0 kilobases. Therefore, a single vector genome may include up to or greater than 10,000 base pairs of nucleotides. In some cases, a vector dose may be about $1 \times 10^6$, $2 \times 10^6$, $3 \times 10^6$, $4 \times 10^6$, $5 \times 10^6$, $6 \times 10^6$, $7 \times 10^6$, $8 \times 10^6$, $9 \times 10^6$, $1 \times 10^7$, $2 \times 10^7$, $3 \times 10^7$, $4 \times 10^7$, $5 \times 10^7$, $6 \times 10^7$, $7 \times 10^7$, $8 \times 10^7$, $9 \times 10^7$, $1 \times 10^8$, $2 \times 10^8$, $3 \times 10^8$, $4 \times 10^8$, $5 \times 10^8$, $6 \times 10^8$, $7 \times 10^8$, $8 \times 10^8$, $9 \times 10^8$, $1 \times 10^9$, $2 \times 10^9$, $3 \times 10^9$, $4 \times 10^9$, $5 \times 10^9$, $6 \times 10^9$, $7 \times 10^9$, $8 \times 10^9$, $9 \times 10^9$, $1 \times 10^{10}$, $2 \times 10^{10}$, $3 \times 10^{10}$, $4 \times 10^{10}$, $5 \times 10^{10}$, $6 \times 10^{10}$, $7 \times 10^{10}$, $8 \times 10^{10}$, $9 \times 10^{10}$, $1 \times 10^{11}$, $2 \times 10^{11}$, $3 \times 10^{11}$, $4 \times 10^{11}$, $5 \times 10^{11}$, $6 \times 10^{11}$, $7 \times 10^{11}$, $8 \times 10^{11}$, $9 \times 10^{11}$, $1 \times 10^{12}$, $2 \times 10^{12}$, $3 \times 10^{12}$, $4 \times 10^{12}$, $5 \times 10^{12}$, $6 \times 10^{12}$, $7 \times 10^{12}$, $8 \times 10^{12}$, $9 \times 10^{12}$, $1 \times 10^{13}$, $2 \times 10^{13}$, $3 \times 10^{13}$, $4 \times 10^{13}$, $5 \times 10^{13}$, $6 \times 10^{13}$, $7 \times 10^{13}$, $8 \times 10^{13}$, $9 \times 10^{13}$, $1 \times 10^{14}$, $2 \times 10^{14}$, $3 \times 10^{14}$, $4 \times 10^{14}$, $5 \times 10^{14}$, $6 \times 10^{14}$, $7 \times 10^{14}$, $8 \times 10^{14}$, $9 \times 10^{14}$, $1 \times 10^{15}$, $2 \times 10^{15}$, $3 \times 10^{15}$, $4 \times 10^{15}$, $5 \times 10^{15}$, $6 \times 10^{15}$, $7 \times 10^{15}$, $8 \times 10^{15}$, $9 \times 10^{15}$, $1 \times 10^{16}$, $2 \times 10^{16}$, $3 \times 10^{16}$, $4 \times 10^{16}$, $5 \times 10^{16}$, $6 \times 10^{16}$, $7 \times 10^{16}$, $8 \times 10^{16}$, $9 \times 10^{16}$, $1 \times 10^{17}$, $2 \times 10^{17}$, $3 \times 10^{17}$, $4 \times 10^{17}$, $5 \times 10^{17}$, $6 \times 10^{17}$, $7 \times 10^{17}$, $8 \times 10^{17}$, $9 \times 10^{17}$, $1 \times 10^{18}$, $2 \times 10^{18}$, $3 \times 10^{18}$, $4 \times 10^{18}$, $5 \times 10^{18}$, $6 \times 10^{18}$, $7 \times 10^{18}$, $8 \times 10^{18}$, $9 \times 10^{18}$, $1 \times 10^{19}$, $2 \times 10^{19}$, $3 \times 10^{19}$, $4 \times 10^{19}$, $5 \times 10^{19}$, $6 \times 10^{19}$, $7 \times 10^{19}$, $8 \times 10^{19}$, $9 \times 10^{19}$, $1 \times 10^{20}$, $2 \times 10^{20}$, $3 \times 10^{20}$, $4 \times 10^{20}$, $5 \times 10^{20}$, $6 \times 10^{20}$, $7 \times 10^{20}$, $8 \times 10^{20}$, $9 \times 10^{20}$ or more vector genome units.

**[0337]** In particular embodiments, a vector contemplated herein is administered to a subject at a titer of at least about $1 \times 10^9$ genome particles/mL, at least about $1 \times 10^{10}$ genome particles/mL, at least about $5 \times 10^{10}$ genome particles/mL, at least about $1 \times 10^{11}$ genome particles/mL, at least about $5 \times 10^{11}$ genome particles/mL, at least about $1 \times 10^{12}$ genome particles/mL, at least about $5 \times 10^{12}$ genome particles/mL, at least about $6 \times 10^{12}$ genome particles/mL, at least about $7 \times 10^{12}$ genome particles/mL, at least about $8 \times 10^{12}$ genome particles/mL, at least about $9 \times 10^{12}$ genome particles/mL, at least about $10 \times 10^{12}$ genome particles/mL, at least about $15 \times 10^{12}$ genome particles/mL, at least about $20 \times 10^{12}$ genome particles/mL, at least about $25 \times 10^{12}$ genome particles/mL, at least about $50 \times 10^{12}$ genome particles/mL, or at least about $100 \times 10^{12}$ genome particles/mL. The terms “genome particles (gp)” or “genome equivalents” or “genome copies” (gc) as used in reference to a viral titer, refer to the number of virions containing the recombinant AAV DNA genome, regardless of infectivity or functionality. The number of genome particles in a particular vector preparation can be measured by well understood methods in the art, for example, quantitative PCR of genomic DNA or for example, in Clark et al. (1999) Hum. Gene Ther., 10:1031-1039; Veldwijk et al. (2002) Mol. Ther., 6:272-278.

**[0338]** A vector of the disclosure may be administered in a volume of fluid. In some cases, a vector may be administered in a volume of about 0.1mL, 0.2mL, 0.3mL, 0.4mL, 0.5mL, 0.6mL, 0.7mL, 0.8mL, 0.9mL, 1.0mL, 2.0mL, 3.0mL, 4.0mL, 5.0mL, 6.0mL, 7.0mL, 8.0mL, 9.0mL, 10.0mL, 11.0mL, 12.0mL, 13.0mL, 14.0mL, 15.0mL, 16.0mL, 17.0mL, 18.0mL, 19.0mL, 20.0mL or greater than 20.0mL. In some cases, a vector dose may be expressed as a concentration or titer of vector administered to a subject. In this case, a vector dose may be expressed as the number of vector genome units per volume (i.e., genome units/volume).

**[0339]** In particular embodiments, a vector contemplated herein is administered to a subject at a titer of at least about $5 \times 10^9$ infectious units/mL, at least about $6 \times 10^9$ infectious units/mL, at least about $7 \times 10^9$ infectious units/mL, at least about $8 \times 10^9$ infectious units/mL, at least about $9 \times 10^9$ infectious units/mL, at least about $1 \times 10^{10}$ infectious units/mL, at least about $1.5 \times 10^{10}$ infectious units/mL, at least about $2 \times 10^{10}$ infectious units/mL, at least about $2.5 \times 10^{10}$ infectious units/mL, at least about $5 \times 10^{10}$ infectious units/mL, at least about $1 \times 10^{11}$ infectious units/mL, at least about $2.5 \times 10^{11}$ infectious units/mL, at least about $5 \times 10^{11}$ infectious units/mL, at least about $1 \times 10^{12}$ infectious units/mL, at least about $2.5 \times 10^{12}$ infectious units/mL, at least about $5 \times 10^{12}$ infectious units/mL, at least about $1 \times 10^{13}$ infectious units/mL, at least about $5 \times 10^{13}$ infectious units/mL, at least about $1 \times 10^{14}$ infectious units/mL. The terms "infection unit (iu)," "infectious particle," or "replication unit," as used in reference to a viral titer, refer to the number of infectious and replication-competent recombinant AAV vector particles as measured by the infectious center assay, also known as replication center assay, as described, for example, in McLaughlin et al. (1988) J. Virol., 62:1963-1973.

**[0340]** In particular embodiments, a vector contemplated herein is administered to a subject at a titer of at least about $5 \times 10^{10}$ transducing units/mL, at least about $1 \times 10^{11}$ transducing units/mL, at least about $2.5 \times 10^{11}$ transducing units/mL, at least about $5 \times 10^{11}$ transducing units/mL, at least about $1 \times 10^{12}$ transducing units/mL, at least about $2.5 \times 10^{12}$ transducing units/mL, at least about $5 \times 10^{12}$ transducing units/mL, at least about $1 \times 10^{13}$ transducing units/mL, at least about $5 \times 10^{13}$ transducing units/mL, at least about $1 \times 10^{14}$ transducing units/mL. The term "transducing unit" (tu)" as used in reference to a viral titer, refers to the number of infectious recombinant AAV vector particles that result in the production of a functional transgene product as measured in functional assays such as described in, for example, in Xiao et al. (1997) Exp. Neurobiol., 144:113-124; or in Fisher et al. (1996) J. Virol., 70:520-532 (LFU assay).

**[0341]** The vector dose will generally be determined by the route of administration. In a particular example, an intraganglionic injection may include from about 1 x $10^9$ to about 1 x $10^{13}$ vector genomes in a volume from about 0.1mL to about 1.0mL. In another particular case, an intrathecal injection may include from about 1 x $10^{10}$ to about 1 x $10^{15}$ vector genomes in a volume from about 1.0 mL to about 12.0 mL. In yet another particular case, an intracranial injection may include from about 1 x $10^9$ to about 1 x $10^{13}$ vector genomes in a volume from about 0.1 mL to about 1.0 mL. In another particular case, an intraneural injection may include from about 1 x $10^9$ to about 1 x $10^{13}$ vector genomes in a volume from about 0.1 mL to about 1.0 mL. In another particular example, an intraspinal injection may include from about 1 x $10^9$ to about 1 x $10^{13}$ vector genomes in a volume from about 0.1 mL to about 1.0 mL. In yet another particular case, a cisterna magna infusion may include from about 5 x $10^9$ to about 5 x $10^{13}$ vector genomes in a volume from about 0.5 mL to about 5.0 mL. In yet another particular case, a subcutaneous injection may include from about 1 x $10^9$ to about 1 x $10^{13}$ vector genomes in a volume from about 0.1mL to about 1.0mL.

**[0342]** In some cases, a vector is delivered to a subject by infusion. A vector dose delivered to a subject by infusion can be measured as a vector infusion rate. Non-limiting examples of vector infusion rates include: 1-10 μL/min for intraganglionic, intraspinal, intracranial or intraneural administration; and 10-1000 μL/min for intrathecal or cisterna magna administration. In some cases, the vector is delivered to a subject by MRI-guided Convection Enhanced Delivery (CED). This technique enables increased viral spread and transduction distributed throughout large volumes of the brain, as well as reduces reflux of the vector along the needle path.

**[0343]** In various embodiments, a method is provided comprising administering a vector encoding a engineered receptor, that deactivates or hyperpolarizes neuronal cells, to one or more neuronal cells that increase pain sensation or sensitivity to pain, and administering a ligand that specifically binds the neuronal cell expressing the engineered receptor to the subject, thereby deactivating the cell, decreasing the sensitivity to pain and potentiating an analgesic effect.

**[0344]** In various embodiments, a method is provided comprising administering a vector encoding a engineered receptor, that activates or polarizes neuronal cells, to one or more neuronal cells that decrease pain sensation or sensitivity to pain, and administering a ligand that specifically binds the neuronal cell expressing the engineered receptor to the subject,

thereby activating the cell, decreasing the sensitivity to pain and potentiating an analgesic effect.

**[0345]** Formulations of ligands may be administered to a subject by various routes. Non-limiting examples of methods of administration include subcutaneous administration, intravenous administration, intramuscular administration, transdermal administration, intradermal administration, intraperitoneal administration, oral administration, infusion, intracranial administration, intrathecal administration, intranasal administration, intraganglionic administration, and intraneural administration. In some cases, administration can involve injection of a liquid formulation of the ligand. In other cases, administration can involve oral delivery of a solid formulation of the ligand. In a particular case, a ligand is administered by oral administration (*e.g.*, a pill, tablet, capsule and the like). In some cases, the oral composition can be administered with food. In another particular case, a ligand is administered by intrathecal injection (i.e., into the subarachnoid space of the spinal cord) for delivery to the cerebrospinal fluid (CSF) of the subject. In another particular case, a ligand is administered topically (*e.g.*, dermal patch, cream, lotion, ointment and the like).

**[0346]** The dosages of the ligands administered to a subject are not subject to absolute limits, but will depend on the nature of the composition and its active ingredients and its unwanted side effects (*e.g.,* immune response against the antibody), the subject being treated and the type of condition being treated and the manner of administration. Generally, the dose will be a therapeutically effective amount, such as an amount sufficient to achieve a desired biological effect, for example an amount that is effective to decrease or attenuate the level of pain experienced by the subject. In particular embodiments, the dose can also be a prophylactic amount or an effective amount. A therapeutically effective amount of ligand may depend on the route of administration, the indication being treated, and/or the ligand selected for use.

**[0347]** In one embodiment, the ligand is first administered to the subject prior to administration of the vector. A therapeutically effective amount of ligand may be administered to a subject at some time after delivery of a vector. Generally, after delivery of a vector, there will be a period of time required for one or more cells of the subject to generate a protein (i.e., engineered receptor) encoded by the vector. During this period of time, administration of a ligand to the subject may not be beneficial to the subject. In this situation, it may be suitable to administer the ligand after an amount of engineered receptor has been produced by one or more cells of the subject.

**[0348]** In one embodiment, the ligand is first administered to the subject at about the same time that the vector is administered to the subject.

**[0349]** In one embodiment, the ligand is first administered 1, 2, 3, 4, 5, 6, 7, 8, 9, 11, or 12 hours, days, weeks, months, or years after administration of the vector to the subject. In some cases, a therapeutically effective amount of a ligand may be administered to a subject at least one day, two days, three days, four days, five days, six days, seven days, eight days, nine days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, 21 days, 22 days, 23 days, 24 days, 25 days, 26 days, 27 days, 28 days, 29 days, 30 days or more than 30 days after delivery of the vector. In a particular example, a therapeutically effective amount of a ligand is administered to a subject at least one week after delivery of a vector. In a further example, the therapeutically effective amount of ligand is administered to the subject daily for at least three consecutive days.

**[0350]** A therapeutically effective amount or dose of a ligand of the disclosure can be expressed as mg or μg of the ligand per kg of subject body mass. In some instances, a therapeutically effective amount of a ligand may be about 0.001 μg/kg, about 0.005 μg/kg, about 0.01 μg/kg, about 0.05 μg/kg, about 0.1 μg/kg, about 0.5 μg/kg, about 1 μg/kg, about 2 μg/kg, about 3 μg/kg, about 4 μg/kg, about 5 μg/kg, about 6 μg/kg, about 7 μg/kg, about 8 μg/kg, about 9 μg/kg, about 10 μg/kg, about 20 μg/kg, about 30 μg/kg, about 40 μg/kg, about 50 μg/kg, about 60 μg/kg, about 70 μg/kg, about 80 μg/kg, about 90 μg/kg, about 100 μg/kg, about 120 μg/kg, about 140 μg/kg, about 160 μg/kg, about 180 μg/kg, about 200 μg/kg, about 220 μg/kg, about 240 μg/kg, about 260 μg/kg, about 280 μg/kg, about 300 μg/kg, about 320 μg/kg, about 340 μg/kg, about 360 μg/kg, about 380 μg/kg, about 400 μg/kg, about 420 μg/kg, about 440 μg/kg, about 460 μg/kg, about 480 μg/kg, about 500 μg/kg, about 520 μg/kg, about 540 μg/kg, about 560 μg/kg, about 580 μg/kg, about 600 μg/kg, about 620 μg/kg, about 640 μg/kg, about 660 μg/kg, about 680 μg/kg, about 700 μg/kg, about 720 μg/kg, about 740 μg/kg, about 760 μg/kg, about 780 μg/kg, about 800 μg/kg, about 820 μg/kg, about 840 μg/kg, about 860 μg/kg, about 880 μg/kg, about 900 μg/kg, about 920 μg/kg, about 940 μg/kg, about 960 μg/kg, about 980 μg/kg, about 1 mg/kg, about 2 mg/kg, about 3 mg/kg, about 4 mg/kg, about 5 mg/kg, about 6 mg/kg, about 7 mg/kg, about 8 mg/kg, about 9 mg/kg, about 10 mg/kg, or greater than 10 mg/kg.

**[0351]** In particular embodiments, the dose of ligand administered to a subject is at least about 0.001 micrograms per kilogram (μg/kg), at least about 0.005 μg/kg, at least about 0.01 μg/kg, at least about 0.05 μg/kg, at least about 0.1 μg/kg, at least about 0.5 μg/kg, 0.001 milligrams per kilogram (mg/kg), at least about 0.005 mg/kg, at least about 0.01 mg/kg, at least about 0.05 mg/kg, at least about 0.1 mg/kg, at least about 0.5 mg/kg , at least about 1 mg/kg,

at least about 2 mg/kg, at least about 3 mg/kg, at least about 4 mg/kg, at least about 5 mg/kg, at least about 5 mg/kg, at least about 6 mg/kg, at least about 7 mg/kg, at least about 8 mg/kg, at least about 8 mg/kg, at least about 9 mg/kg, or at least about 10 or more mg/kg.

**[0352]** In particular embodiments, the dose of ligand administered to a subject is at least about 0.001 μg/kg to at least about 10 mg/kg, at least about 0.01 μg/kg to at least about 10 mg/kg, at least about 0.1 μg/kg to at least about 10 mg/kg, at least about 1 μg/kg to at least about 10 mg/kg, at least about 0.01 mg/kg to at least about 10 mg/kg, at least about 0.1 mg/kg to at least about 10 mg/kg, or at least about 1 mg/kg to at least about 10 mg/kg, or any intervening range thereof.

**[0353]** In some aspects, a therapeutically effective amount of a ligand can be expressed as a molar concentration (i.e., M or mol/L). In some cases, a therapeutically effective amount of a ligand can be about 1nM, 2nM, 3nM, 4nM, 5nM, 6nM, 7nM, 8nM, 9nM, 10nM, 20nM, 30nM, 40nM, 50nM, 60nM, 70nM, 80nM, 90nM, 100nM, 200nM, 300nM, 400nM, 500nM, 600nM, 700nM, 800nM, 900nM, 1mM, 2mM, 3mM, 4mM, 5mM, 6mM, 7mM, 8mM, 9mM, 10mM, 20mM, 30mM, 40mM, 50mM, 60mM, 70mM, 80mM, 90mM, 100mM, 200mM, 300mM, 400mM, 500mM, 600mM, 700mM, 800mM, 900mM, 1000mM or greater.

**[0354]** A therapeutically effective amount of a ligand can be administered once or more than once each day. In some cases, a therapeutically effective amount of a ligand is administered as needed (*e.g.*, when pain relief is needed). The ligand may be administered serially (*e.g.*, every day without a break for the duration of the treatment regimen). In some cases, the treatment regimen can be less than a week, a week, two weeks, three weeks, a month, or greater than a month. In some cases, a therapeutically effective amount of a ligand is administered for a day, at least two consecutive days, at least three consecutive days, at least four consecutive days, at least five consecutive days, at least six consecutive days, at least seven consecutive days, at least eight consecutive days, at least nine consecutive days, at least ten consecutive days, or at least greater than ten consecutive days. In a particular case, a therapeutically effective amount of a ligand is administered for three consecutive days. In some cases, a therapeutically effective amount of a ligand can be administered one time per week, two times per week, three times per week, four times per week, five times per week, six times per week, seven times per week, eight times per week, nine times per week, 10 times per week, 11 times per week, 12 times per week, 13 times per week, 14 times per week, 15 times per week, 16 times per week, 17 times per week, 18 times per week, 19 times per week, 20 times per week, 25 times per week, 30 times per week, 35 times per week, 40 times per week, or greater than 40 times per week. In some cases, a therapeutically effective amount of a ligand

can be administered one time per day, two times per day, three times per day, four times per day, five times per day, six times per day, seven times per day, eight times per day, nine times per day, 10 times per day, or greater than 10 times per day. In some cases, a therapeutically effective amount of a ligand is administered at least every hour, at least every two hours, at least every three hours, at least every four hours, at least every five hours, at least every six hours, at least every seven hours, at least every eight hours, at least every nine hours, at least every 10 hours, at least every 11 hours, at least every 12 hours, at least every 13 hours, at least every 14 hours, at least every 15 hours, at least every 16 hours, at least every 17 hours, at least every 18 hours, at least every 19 hours, at least every 20 hours, at least every 21 hours, at least every 22 hours, at least every 23 hours, or at least every day. The dose of ligand may be administered to the subject continuously, or 1, 2, 3, 4, or 5 times a day; 1, 2, 3, 4, 5, 6, or 7 times a week, 1, 2, 3, or 4 times a month, once every 2, 3, 4, 5, or 6 months, or once a year, or at even longer intervals. The duration of treatment can last a day, 1, 2, or 3 weeks, 1, 2, 3, 4, 5, 7, 8, 9, 10, or 11 months, 1, 2, 3, 4, 5, or more years, or longer.

**[0355]** A subject treated by methods and compositions disclosed herein can be a human, or can be a non-human animal. The term "treat" and its grammatical equivalents used herein generally refer to the use of a composition or method to reduce, eliminate, or prevent symptoms of a disease and includes achieving a therapeutic benefit and/or a prophylactic benefit. By therapeutic benefit is meant slowing the progression of, halting the progression of, reversing the progression of, or eradication or amelioration of the symptoms of the disorder or condition being treated. A prophylactic benefit of treatment includes reducing the risk of a condition, retarding the progress of a condition, or decreasing the likelihood of occurrence of a condition.

**[0356]** Non-limiting examples of non-human animals include a non-human primate, a livestock animal, a domestic pet, and a laboratory animal. For example, a non-human animal can be an ape (*e.g.*, a chimpanzee, a baboon, a gorilla, or an orangutan), an old world monkey (*e.g.*, a rhesus monkey), a new world monkey, a dog, a cat, a bison, a camel, a cow, a deer, a pig, a donkey, a horse, a mule, a lama, a sheep, a goat, a buffalo, a reindeer, a yak, a mouse, a rat, a rabbit, or any other non-human animal. The compositions and methods as described herein are amenable to the treatment of a veterinary animal. A veterinary animal can include, without limitation, a dog, a cat, a horse, a cow, a sheep, a mouse, a rat, a guinea pig, a hamster, a rabbit, a snake, a turtle, and a lizard. In some aspects, contacting the tissue or cell population with a composition comprises administering the composition to a cell population or subject. In some embodiments, administration occurs *in vitro*, for example by adding the composition to

a cell culture system. In some aspects, administration occurs *in vivo*, for example by administration through a particular route. Wherein more than one composition is to be administered, the compositions may be administered via the same route at the same time (*e.g.*, on the same day), or via the same route at different times. Alternatively, the compositions may be administered via different routes at the same time (*e.g.*, on the same day) or via different routes at different times.

**[0357]** The number of times a composition is administered to a subject in need thereof depends on the discretion of a medical professional, the disorder, the severity of the disorder, and the subject's response to the formulation. In some aspects, administration of a composition occurs at least once. In further aspects, administration occurs more than once, for example 2, 3, 4, 5, 6, 7, 8, 9, 10 or more times in a given period. The dosage of each administration and/or frequency of administrations may be adjusted as necessary based on the patient's condition and physiologically responses.

**[0358]** In some embodiments, compositions may be administered a sufficient amount of times to achieve a desired physiologic effect or improvement in a subject's condition. In the case wherein the subject's condition does not improve, upon the doctor's discretion the composition may be administered chronically, that is, for an extended period of time, including throughout the duration of the subject's life in order to ameliorate or otherwise control or limit the symptoms of the subject's disease or condition. In the case wherein the subject's status does improve, upon the doctor's discretion the composition may administered continuously; alternatively, the dose of drug being administered may be temporarily reduced or temporarily suspended for a certain length of time (*i.e.*, a "drug holiday"). The length of the drug holiday varies between 2 days and 1 year, including by way of example only, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 10 days, 12 days, 15 days, 20 days, 28 days, 35 days, 50 days, 70 days, 100 days, 120 days, 150 days, 180 days, 200 days, 250 days, 280 days, 300 days, 320 days, 350 days, and 365 days. The dose reduction during a drug holiday may be from 10%- 100%, including by way of example only 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, and 100%.

**[0359]** Where compositions are administered more than once, each administration may be performed by the same actor and/or in the same geographical location. Alternatively, each administration may be performed by a different actor and/or in a different geographical location.

**[0360]** With regard to human and veterinary treatment, the amount of a particular ligand(s) that is administered may be dependent on a variety of factors, including the disorder

being treated and the severity of the disorder; activity of the specific ligand(s) employed; the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific ligand(s) employed; the duration of the treatment; drugs used in combination or coincidental with the specific ligand(s) employed; the judgment of the prescribing physician or veterinarian; and like factors known in the medical and veterinary arts. Similarly, the effective concentration of a given composition may be dependent on a variety of factors including the age, sex, weight, genetic status, and overall health of the patient or subject.

**Kits**

**[0361]** In one aspect, the present disclosure provides kits comprising a vector comprising a polynucleotide encoding an engineered receptor of the disclosure. In one aspect, the present disclosure provides kits comprising the engineered receptor of the disclosure.

**[0362]** In some embodiments, the kit comprises (a) a vector comprising a polynucleotide encoding an engineered receptor of the disclosure; and (b) a non-native ligand of the disclosure. In some embodiments, the vector is a viral vector. In some embodiments, the vector is an AAV vector. In some embodiments, the kit comprises instructions for administering the vector. In some embodiments, the kit comprises a device adapted to administration of the vector.

**[0363]** In some embodiments, the kit comprises (a) an engineered receptor of the disclosure; and (b) a non-native ligand of the disclosure.

**[0364]** Exemplary combinations of engineered receptors described herein and non-native ligands that can comprise the kits of the present disclosure are provided in Table 29 below. Each of the engineered receptors in Table 29 may be present as a protein, a polynucleotide encoding the protein, or a vector comprising the polynucleotide encoding the protein. In some embodiments, the engineered receptor comprises a ligand binding domain derived from human α7-nAChR. In some embodiments, the engineered receptor comprises an ion pore domain derived from human Glycine receptor. In some embodiments, the human Glycine receptor is human Glycine receptor α1. In some embodiments, the engineered receptor comprises a polypeptide sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 33 except for the indicated mutation in Table 29.

**Table 29: Exemplary Kits**

| Engineered Receptor with Amino Acid Substitution(s) Corresponding to the Indicated Position(s) in SEQ ID NO: 4 | Non-native ligand | Non-limiting Example of the Engineered Receptor |
|---|---|---|
| L131S_S172D | CNL001 | CODA534 |
| L131T_S172D | CNL001 | CODA535 |
| L131D_S172D | CNL002 | CODA536 |
| L131D_S172D | AZD-0328 | CODA536 |
| L131D_S172D | Facinicline | CODA536 |
| Y115D_S170T | Facinicline | CODA805 |
| Y115D_S170T | TC-6987 | CODA805 |
| Y115D_L131Q | AZD-0328 | CODA806 |
| Y115D_L131Q | Facinicline | CODA806 |
| Y115D_L131Q | TC-6987 | CODA806 |
| Y115D_L131E | TC-5619 | CODA807 |
| Y115D_L131E | AZD-0328 | CODA807 |
| Y115D_L131E | Facinicline | CODA807 |
| Y115D_L131E | TC-6987 | CODA807 |
| Y140I | CNL001 | CODA952 |
| Y140I | TC-5619 | CODA952 |
| Y140I | CNL002 | CODA952 |
| Y140I | Facinicline | CODA952 |
| Y140C | CNL001 | CODA965 |
| Y140C | TC-5619 | CODA965 |
| Y140C | CNL002 | CODA965 |
| Y140C | ABT-126 | CODA965 |
| Y140C | TC-6987 | CODA965 |
| R101F_L131G | CNL001 | CODA1025 |
| R101F_L131G | TC-5619 | CODA1025 |
| R101F_L131G | CNL002 | CODA1025 |
| R101F_L131G | AZD-0328 | CODA1025 |
| R101F_L131G | TC-6987 | CODA1025 |
| R101F_L131G | Varenicline | CODA1025 |
| R101F_L131D | CNL001 | CODA1027 |
| R101F_L131D | TC-5619 | CODA1027 |
| R101F_L131D | CNL002 | CODA1027 |
| R101F_L131D | TC-6987 | CODA1027 |
| Y115E_Y210W | TC-5619 | CODA1039 |
| Y115E_Y210W | CNL002 | CODA1039 |
| Y115E_Y210W | ABT-126 | CODA1039 |
| R101W_Y210V | TC-5619 | CODA1045 |
| R101F_Y210V | TC-5619 | CODA1047 |
| R101F_Y210F | CNL001 | CODA1048 |
| R101F_Y210F | TC-5619 | CODA1048 |
| R101M_L131A | CNL001 | CODA1053 |
| R101M_L131A | TC-5619 | CODA1053 |
| R101M_L131A | Varenicline | CODA1053 |
| R101M_L131F | CNL001 | CODA1054 |
| R101M_L131F | TC-5619 | CODA1054 |
| R101M_L131F | Varenicline | CODA1054 |
| R101W_Y115E_Y210W | TC-5619 | CODA1055 |
| R101F_Y115E_Y210W | TC-5619 | CODA1056 |

| Engineered Receptor with Amino Acid Substitution(s) Corresponding to the Indicated Position(s) in SEQ ID NO: 4 | Non-native ligand | Non-limiting Example of the Engineered Receptor |
|---|---|---|
| W77F_R101F_L131D | CNL001 | CODA1138 |
| W77F_R101F_L131D | CNL002 | CODA1138 |
| W77F_R101F_L131D | ABT-126 | CODA1138 |
| R101F_L131N_S172D | CNL001 | CODA1140 |
| R101F_L131N_S172D | CNL002 | CODA1140 |
| Q139E_S172D | CNL001 | CODA1157 |
| Q139E_S172D | CNL002 | CODA1157 |
| S172D_Y210W | CNL001 | CODA1173 |
| S172D_Y210W | CNL002 | CODA1173 |
| S172D_Y210W | ABT-126 | CODA1173 |
| L131S_S172D | CNL001 | CODA534 |
| L131T_S172D | CNL001 | CODA535 |
| L131D_S172D | CNL002 | CODA536 |
| Y140R | TC-5619 | CODA967 |
| Y115E_L131M | ABT-126 | CODA1101 |
| Q139W_S172D | ABT-126 | CODA1167 |
| Q139W_S172D | CNL002 | CODA1167 |
| Q139W_Y210W | ABT-126 | CODA1168 |
| Q139W_S172D_Y217I | ABT-126 | CODA1245 |
| Q139W_S172D_Y217I | CNL001 | CODA1245 |
| Y115E_Q139E_S172D | CNL001 | CODA1261 |
| R101F_L131G_Q139L_Y217F | Varenicline | CODA1458 |
| Y115Q_S172D | CNL001 | CODA1471 |
| L141M_S172D_Y210W | CNL001 | CODA1500 |
| L141M_S172D_Y210W | CNL002 | CODA1500 |
| R101F_L131N_Y217F | Varenicline | CODA1507 |
| R101F_L131N_Y217F | CNL002 | CODA1507 |

**[0365]** In some embodiments, the kit further comprises packaging material and one or more components therein. A kit may include a label or packaging insert including a description of the components or instructions for use in vitro, in vivo, or ex vivo, of the components therein.

**[0366]** Labels or inserts can include identifying information of one or more components therein, dose amounts, clinical pharmacology of the active ingredient(s) including mechanism of action, pharmacokinetics and pharmacodynamics. Labels or inserts can include information identifying the manufacturer, lot numbers, manufacturer location and date, expiration dates. Labels or inserts can include information identifying manufacturer information, lot numbers, manufacturer location and date. Labels or inserts can include information on a disease a kit component may be used for. Labels or inserts can include instructions for the clinician or subject for using one or more of the kit components in a method, use, or treatment protocol or therapeutic regimen. Instructions can include dosage amounts,

frequency or duration, and instructions for practicing any of the methods, uses, treatment protocols or prophylactic or therapeutic regimes described herein.

**[0367]** Labels or inserts can include information on any benefit that a component may provide, such as a prophylactic or therapeutic benefit. Labels or inserts can include information on potential adverse side effects, complications or reactions, such as warnings to the subject or clinician regarding situations where it would not be appropriate to use a particular composition. Adverse side effects or complications could also occur when the subject has, will be or is currently taking one or more other medications that may be incompatible with the composition, or the subject has, will be or is currently undergoing another incompatible treatment protocol or therapeutic regimen and, therefore, instructions could include information regarding such incompatibilities.

**[0368]** All papers, publications and patents cited in this specification are herein incorporated by reference as if each individual paper, publication or patent were specifically and individually indicated to be incorporated by reference and are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited. However, mention of any reference, article, publication, patent, patent publication, and patent application cited herein is not, and should not be taken as an acknowledgment or any form of suggestion that they constitute valid prior art or form part of the common general knowledge in any country in the world.

**[0369]** Unless the context indicates otherwise, it is specifically intended that the various features described herein can be used in any combination.

**[0370]** Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs.

**[0371]** It is to be understood that the description above as well as the examples that follow are intended to illustrate, and not limit, the scope of the invention. Other aspects, advantages and modifications within the scope of the invention will be apparent to those skilled in the art to which the invention pertains.

## EXAMPLES

### Example 1. Discovery of Engineered Receptors Comprising Mutations in the Ligand Binding Domain

**[0372]** To generate LGICs that conduct anion current following exposure to non-native small molecule agonists of the human α7-nAChR, chimeric ligand gated ion channel (LGIC) receptors comprising the ligand binding domain derived from the human α7 nicotinic acetylcholine receptor (α7-nAChR) and the chloride-conducting ion pore domain derived from the human GlyR1α were genetically engineered. An engineered receptor with an amino acid sequence of SEQ ID NO: 33 (CODA71) was identified, which was approximately as sensitive to acetylcholine, ABT-126 and TC-6987 as wild type α7-nAChR, with TC-6987 showing partial agonist activity on SEQ ID NO:33 similar to wild type. SEQ ID NO:33 was approximate 2-fold less sensitive to nicotine and approximately 3-fold and 10-fold more sensitive to AZD-0328 and Facinicline/RG3487, respectively, than wild type. CODA71 is described in detail in WO2019104307 and WO2021035179, which are incorporated herein in their entireties.

**[0373]** Amino acid substitutions were introduced into the ligand binding domain of the engineered receptor with the amino acid sequence of SEQ ID NO: 33. The binding pocket for each ligand in the α7-nAChR was modeled, and the amino acid residues that form the binding pocket were mapped. Libraries of single, double, and triple mutant chimeric LGICs were then generated, each mutant chimeric LGIC comprising substitutions in one or more amino acids of the ligand binding pocket of SEQ ID NO: 33. The parental chimera receptor (SEQ ID NO: 33) was cloned into pcDNA3.1(+) (Invitrogen) using BamHI and EcoRI sites by standard molecular biology techniques. Amino acid substitutions were introduced by site-directed mutagenesis. A list of the mutants generated is provided above in Table 20.

**[0374]** All of the resulting engineered receptors were analyzed for their potency to the native ligand, acetylcholine (Ach), and non-native ligands such as, AZD-0328 (adisinsight.springer.com/drugs/800018503), TC-6987 (drugbank.ca/drugs/DB14854), ABT-126 (medchemexpress.com/Nelonicline.html), TC-5619 (en.wikipedia.org/wiki/Bradanicline), TC-6683 (pubchem.ncbi.nlm.nih.gov/compound/TC-6683_-Azd1446), Varenicline (en.wikipedia.org/wiki/Varenicline), Facinicline/RG3487 (researchgate.net/figure/Molecular-structure-of-RG3487_fig1_47499934), CNL001, and CNL002.

**Example 2: Characterization of the Engineered Receptors using High Throughput Fluorescence Based Plate Screening**

**[0375]** To screen these mutant LGICs for those having novel response profiles to ligands, an anion reporter assay was developed to assess the function of the LGICs in a high throughput format. In this assay, cells expressing a YFP reporter whose fluorescence is quenched in the presence of anion are transfected with DNA encoding the channel of interest. Upon exposure to ligand, channels that are activated will flux anion, resulting in a dose-dependent quench of the YFP that can be detected on a plate reader. The greater the quench signal (*i.e.*, the more positive the value), the higher the ligand activity on the receptor is.

**[0376]** Lenti-X 293T cells (LX293T, Clontech) were maintained in DMEM containing 10% FBS and 1% penicillin/streptomycin (Invitrogen). For plate reader assays, LX293T cells were infected with a lentivirus to create cells that stably express a mutant YFP (H148Q/I152L) reporter, which displays enhanced sensitivity to anions. Two days before assay, cells were split at a density of 20,000 cells/well in a 96-well tissue culture plate coated with poly-d lysine (Thermo Scientific). The next day, the cells were transiently transfected with 0.1 μg of DNA per well using standard Fugene protocol (Promega). On the day of assay, cells were washed 2 times in 1X extracellular solution (1X ECS: 140 mM NaCl, 5 mM KCl, 1 mM MgCl2, 2 mM CaCl2, 10 mM HEPES, 10 mM glucose, pH 7.2, mOsms 300). After the last wash, 100 μL of 1X ECS was added to the wells and the plate was incubated at 37°C for 30 min. While incubating the plate, the drugs were diluted to a 2X concentration in 1X ECS-NaI (same components as 1X ECS except the 140 mM NaCl was replaced by 140 mM NaI). The plates were then read on a Flexstation3 (Molecular Devices). Each well, 8 wells at a time, of the plate is read for 2 min using a Flexstation3 (Molecular Devices) as follows: 1) a baseline YFP fluorescence is read for 17 sec, 2) 100 μL of ligand is added, and 3) the changes in YFP fluorescence are then measured every 1.3 sec for 1 minute and 43 seconds. The percent quench is calculated from the average fluorescence of the last 10 seconds of the read divided by the baseline average of the first 15 seconds prior to the ligand addition.

**[0377]** **FIGs. 1A-1J** provide heat maps of the percent quench of YFP fluorescence following stimulation by various doses of either acetylcholine or the non-native ligand as indicated in the Figures. CODA71 (SEQ ID NO: 33) was used as a control, and CODA75 (SEQ ID NO: 29, a non-responding chimeric engineered receptor) was used as the negative control. Quenching of the fluorescence signal, as indicated by blue shaded cells, indicate the level of engineered receptor activation by the non-native ligand at that concentration. The results

indicate that the engineered receptors have varying potency towards acetylcholine and the non-native ligands tested (see also Section C1 "Amino Acid Mutations" of the disclosure above).

**[0378]** The numeric values in these heat maps can be found in **Tables 11-20** below.

**Table 11: YFP fluorescence quenching using acetylcholine**

| Name | Amino Acid Mutation(s) in SEQ ID NO: 33 | 10 uM ach | 30 uM ach | 50 uM ach | 100 uM ach | 300 uM ach | 500 uM ach | 1 mM ach | 3 mM ach | 5 mM ach | 10 mM ach |
|---|---|---|---|---|---|---|---|---|---|---|---|
| CODA71 | - | 15.61 | 63.31 | 73.16 | 74.94 | 65.94 | 62.78 | 74.24 | 76.27 | 77.15 | 77.96 |
| CODA75 | SEQ ID NO: 29 | -12.88 | -18.67 | -0.29 | -14.46 | -16.31 | -17.41 | -16.46 | -12.38 | -20.88 | -8.69 |
| CODA217 | W77F | -5.86 | | | 70.06 | | | 68.71 | 73.45 | | |
| CODA109 | S170T | 82.91 | 82.53 | 82.11 | 83.68 | | | 69.89 | 78.12 | | |
| CODA236 | R101F | -18.24 | | | 60 | | | 66.07 | 71.38 | | |
| CODA237 | R101M | -16.79 | | | -18.63 | | | 46.28 | 69.31 | | |
| CODA238 | R101W | -16.58 | | | 75.18 | | | 78.99 | 75.74 | | |
| CODA282 | Y115D | 6.81 | | | 1.19 | | | -14.05 | 2.37 | | |
| CODA283 | Y115E_N379K | -6.16 | | | -11.12 | | | -2.74 | 53.61 | | |
| CODA325 | L131G | 5.66 | | | 74.07 | | | 62.65 | 69.49 | | |
| CODA326 | L131A | 7.43 | | | 73.57 | | | 71.8 | 74.49 | | |
| CODA330 | L131F | 11.07 | | | 43.34 | | | 77.52 | 74.89 | | |
| CODA333 | L131S | -10.11 | | | -10.05 | | | 32.11 | 57.37 | | |
| CODA334 | L131Q | -2.63 | 30.06 | | 71.27 | | | 79.66 | 74.9 | | |
| CODA335 | L131T | -9.13 | | | -9.29 | | | 0.57 | 35.37 | | |
| CODA339 | L131D | 4.56 | | | 10.19 | | | 77.34 | 77.79 | | |
| CODA340 | L131E | 15.29 | 66.56 | | 81.31 | | | 80.77 | 77.31 | | |
| CODA342 | L131R | -2.57 | | | -3.18 | | | 78.98 | 75.75 | | |
| CODA337 | L131N | 28.32 | | | 81.8 | | | 71.22 | 73.7 | | |
| CODA945 | Q139E | -1.25 | | | 71.21 | | | 71.79 | 76.04 | | |
| CODA377 | S172D | -3.19 | | | 0.07 | | | 71.7 | 76.95 | | |

| Name | Amino Acid Mutation(s) in SEQ ID NO: 33 | 10 uM ach | 30 uM ach | 50 uM ach | 100 uM ach | 300 uM ach | 500 uM ach | 1 mM ach | 3 mM ach | 5 mM ach | 10 mM ach |
|---|---|---|---|---|---|---|---|---|---|---|---|
| CODA405 | Y210V | -6.31 | | | -14.48 | | | -2.82 | 1.06 | | |
| CODA407 | Y210F | -3.75 | | | -1.6 | | | 71.65 | 79.09 | | |
| CODA409 | Y210W | -5.43 | | | 17.14 | | | 78.84 | 78.55 | | |
| CODA534 | L131S_S172D | -11.3 | | | -27.53 | | | 2.84 | 14.65 | | |
| CODA535 | L131T_S172D | -12.24 | | | -26.23 | | | -1.16 | 2.97 | | |
| CODA536 | L131D_S172D | -12.91 | | | -20.88 | | | 79.85 | 79.98 | | |
| CODA805 | Y115D_S170T | -0.46 | | | -4.93 | | | -11.18 | -3.53 | | |
| CODA806 | Y115D_L131Q | 9.47 | | | 6.12 | | | -1.56 | 17.57 | | |
| CODA807 | Y115D_L131E | 15.45 | | | 13.77 | | | 27.42 | 53.62 | | |
| CODA952 | Y140I | -1.97 | | | 2.79 | | | 58.81 | 66.9 | | |
| CODA965 | Y140C | -21.71 | | | 3.49 | | | 63.34 | 51.92 | | |
| CODA1025 | R101F_L131G | 12.57 | | -3.46 | 12.65 | -6.96 | -6.77 | 19.42 | 65.06 | | |
| CODA1027 | R101F_L131D | -1.62 | | -1.6 | -2.62 | -6.12 | -7.71 | -5.73 | 33.69 | | |
| CODA1039 | Y115E_Y210W | -2.97 | | -3.94 | 0.35 | -4.13 | -5.18 | 1.21 | 26.69 | | |
| CODA1045 | R101W_Y210V | -2.52 | | | -11.98 | -16.71 | -12.87 | -14.41 | -7.4 | | -1.5 |
| CODA1047 | R101F_Y210V | -4.26 | | | -13.05 | -11.73 | -10.75 | -8.81 | -6.77 | | -1.03 |
| CODA1048 | R101F_Y210F | | | | -17.31 | -13.7 | -10.1 | 5.53 | 68.61 | | 78.22 |
| CODA1053 | R101M_L131A | -3.98 | | | -11 | -7.92 | 7.36 | 57.28 | 60.54 | | 79.46 |
| CODA1054 | R101M_L131F | -3.98 | | | -10.34 | -10.25 | -11.46 | 1.77 | 36.81 | | 74.02 |
| CODA1055 | R101W_Y115E_Y210W | | | | -15.63 | -12.05 | -12.87 | -11.55 | 12.68 | | 37.58 |
| CODA1056 | R101F_Y115E_Y210W | | | | -20.55 | -19.32 | -19.75 | -14.08 | 14.07 | | 25.7 |
| CODA1138 | W77F_R101F_L131D | -12.92 | -15.36 | | -19.35 | -14.79 | | 37.5 | 61.73 | | |
| CODA1140 | R101F_L131N_S172D | -20.07 | -16.92 | | -25.8 | -11.21 | | 9.02 | 45 | | |

| Name | Amino Acid Mutation(s) in SEQ ID NO: 33 | 10 uM ach | 30 uM ach | 50 uM ach | 100 uM ach | 300 uM ach | 500 uM ach | 1 mM ach | 3 mM ach | 5 mM ach | 10 mM ach |
|---|---|---|---|---|---|---|---|---|---|---|---|
| CODA1157 | Q139E_S172D | -36.89 | -37.78 | | -25.67 | -18.94 | | 16.38 | 45.71 | | |
| CODA1173 | S172D_Y210W | | | | | | | -6.12 | 11.31 | | |

**Table 12: YFP fluorescence quenching using CNL001**

| Name | Amino Acid Mutation(s) in SEQ ID NO: 33 | 1nM CNL001 | 1nM CNL001 | 10nM CNL001 | 10nM CNL001 | 50nM CNL001 | 50nM CNL001 | 100nM CNL001 | 100nM CNL001 | 1uM CNL001 | 1uM CNL001 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| CODA71 | - | 3.727 | 20.231 | 81.096 | 82.368 | 83.62 | 84.482 | 84.297 | 85.368 | 85.647 | 86.358 |
| CODA75 | SEQ ID NO: 29 | -8.496 | -2.266 | 1.816 | -4.649 | -5.922 | -5.597 | -8.159 | -5.058 | -4.272 | -1.281 |
| CODA217 | W77F | -22.273 | -19.415 | -9.563 | 6.398 | 61.938 | 66.837 | 79.791 | 80.908 | 83.444 | 83.905 |
| CODA109 | S170T | 19.755 | 65.484 | 79.024 | 81.563 | 81.926 | 83.092 | 83 | 83.811 | 81.834 | 83.858 |
| CODA236 | R101F | 9.048 | 26.601 | 71.533 | 72.948 | 75.895 | 74.238 | 76 | 75.861 | 76.367 | 76.162 |
| CODA237 | R101M | -2.114 | -5.079 | 15.096 | 50.554 | 66.585 | 74.462 | 78 | 77.411 | 79.06 | 79.162 |
| CODA238 | R101W | 32.42 | 54.768 | 75.149 | 75.339 | 75.881 | 77.844 | 78 | 78.575 | 81.126 | 79.482 |
| CODA282 | Y115D | -15.837 | -6.519 | -4.862 | -8.244 | -3.807 | -2.871 | -7.924 | 2.11 | 11.047 | 8.04 |
| CODA283 | Y115E_N379K | -0.713 | -0.621 | 7.739 | 15.277 | 19.489 | 33.703 | 56.886 | 60.171 | 69.836 | 73.855 |
| CODA325 | L131G | 74.429 | 78.715 | 79.033 | 79.322 | 79.966 | 80.091 | 81 | 79.701 | 79.289 | 80.581 |
| CODA326 | L131A | 76.424 | 80.567 | 80.284 | 81.232 | 81.575 | 82.544 | 81 | 81.718 | 82.334 | 81.539 |
| CODA330 | L131F | 76.289 | 78.896 | 79.339 | 80.245 | 80.831 | 80.605 | 81 | 80.663 | 81.293 | 81.428 |
| CODA333 | L131S | 76.165 | 78.463 | 79.349 | 78.238 | 80.221 | 81.789 | 81.855 | 80.766 | 81.707 | 82.665 |
| CODA334 | L131Q | 76.165 | 78.463 | 79.349 | 78.238 | 80.221 | 81.789 | 82 | 80.766 | 81.707 | 82.665 |
| CODA335 | L131T | -9.942 | -0.475 | 17.889 | 38.46 | 61.741 | 69.159 | 73.635 | 76.092 | 80.633 | 79.637 |
| CODA339 | L131D | 73.711 | 76.44 | 77.049 | 77.76 | 77.882 | 78.708 | 79.567 | 79.273 | 79.992 | 79.927 |
| CODA340 | L131E | 76.413 | 77.144 | 78.258 | 78.763 | 79.757 | 79.327 | 79.011 | 79.948 | 79.855 | 80.618 |

| Name | Amino Acid Mutation(s) in SEQ ID NO: 33 | 1nM CNL001 | 1nM CNL001 | 10nM CNL001 | 10nM CNL001 | 50nM CNL001 | 50nM CNL001 | 100nM CNL001 | 100nM CNL001 | 1uM CNL001 | 1uM CNL001 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| CODA342 | L131R | 23.532 | 26.054 | 43.69 | 54.016 | 59.955 | 56.091 | 64.536 | 58.987 | 65.987 | 64.312 |
| CODA337 | L131N | 76.917 | 79.554 | 79.479 | 79.506 | 81.465 | 81.297 | 82.619 | 81.722 | 81.96 | 81.773 |
| CODA945 | Q139E | 6.65 | 13.512 | 78.678 | 82.723 | 84.91 | 84.849 | 85.046 | 84.642 | 85.493 | 86.071 |
| CODA377 | S172D | 67.544 | 74.209 | 74.346 | 75.816 | 77.068 | 76.99 | 79.352 | 77.865 | 80.272 | 79.432 |
| CODA405 | Y210V | -16.246 | -4.217 | -6.142 | -10.003 | -11.054 | -10.06 | -3.832 | -3.784 | 1.129 | 7.1 |
| CODA407 | Y210F | -10.845 | -2.629 | 4.66 | 12.246 | 29.307 | 41.78 | 50.654 | 62.054 | 81.742 | 81.304 |
| CODA409 | Y210W | -8.914 | -14.319 | -1.444 | 4.393 | 11.897 | 11.434 | 12.719 | 16.053 | 14.077 | 15.495 |
| CODA534 | L131S_S172D | 23.438 | 28.59 | 56.882 | 56.777 | 65.044 | 67.373 | 68.666 | 70.357 | 73.019 | 73.527 |
| CODA535 | L131T_S172D | 8.736 | 24.572 | 60.552 | 63.09 | 73.242 | 73.889 | 76.147 | 76.21 | 78.988 | 78.818 |
| CODA536 | L131D_S172D | | | | | | | | | | |
| CODA805 | Y115D_S170T | -10.729 | -15.278 | -20.403 | -14.905 | -14.303 | -15.741 | -12.637 | -10.606 | -13.535 | -11.335 |
| CODA806 | Y115D_L131Q | 6.587 | -5.179 | -2.366 | -1.272 | 2.763 | 1.995 | 3.091 | 1.45 | 7.331 | 9.103 |
| CODA807 | Y115D_L131E | 0.885 | -1.707 | -0.567 | 0.043 | 5.141 | 9.409 | 9.796 | 11.009 | 27.914 | 29.109 |
| CODA952 | Y140I | 0.368 | -1.976 | -3.923 | 4.348 | 74.107 | 75.866 | 80.323 | 81.996 | 83.489 | 82.208 |
| CODA965 | Y140C | -12.765 | -12.608 | 68.134 | 71.394 | 80.298 | 82.159 | 82.404 | 82.14 | 82.34 | 80.649 |
| CODA1025 | R101F_L131G | -18.527 | -21.577 | 14.063 | 49.818 | 77.765 | 79.23 | 80.544 | 80.695 | 81.191 | 82.385 |
| CODA1027 | R101F_L131D | 24.049 | 36.755 | 52.927 | 61.449 | 67.308 | 68.77 | 69.049 | 71.613 | 71.811 | 69.907 |
| CODA1039 | Y115E_Y210W | -1.12 | -3.941 | 6.275 | 11.943 | 12.949 | 18.635 | 17.576 | 18.319 | 19.329 | 20.672 |
| CODA1045 | R101W_Y210V | 0.804 | 11.275 | 15.843 | 16.619 | 15.205 | 18.771 | 9.332 | 18.218 | 35.294 | 36.542 |
| CODA1047 | R101F_Y210V | -7.82 | -8.382 | -5.381 | -1.431 | -0.999 | 3.909 | -0.823 | -5.885 | 1.638 | 3.483 |
| CODA1048 | R101F_Y210F | 9.114 | 0.845 | 28.944 | 53.629 | 68.581 | 74.379 | 80.541 | 80.774 | 83.143 | 81.751 |
| CODA1053 | R101M_L131A | -8.554 | -13.711 | 39.631 | 66.809 | 82.122 | 82.031 | 83.312 | 83.986 | 84.363 | 83.877 |
| CODA1054 | R101M_L131F | 21.932 | 39.689 | 54.192 | 64.111 | 71.409 | 70.989 | 74.148 | 71.722 | 71.378 | 71.411 |

| Name | Amino Acid Mutation(s) in SEQ ID NO: 33 | 1nM CNL001 | 1nM CNL001 | 10nM CNL001 | 10nM CNL001 | 50nM CNL001 | 50nM CNL001 | 100nM CNL001 | 100nM CNL001 | 1uM CNL001 | 1uM CNL001 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| CODA1055 | R101W_Y115E_Y210W | -12.889 | -13.295 | -10.401 | -7.981 | -1.972 | -1.661 | 0.277 | 4.325 | 2.88 | 3.259 |
| CODA1056 | R101F_Y115E_Y210W | -8.104 | -12.153 | -10.462 | -4.735 | -8.617 | -1.606 | -1.243 | 1.838 | -0.567 | 4.663 |
| CODA1138 | W77F_R101F_L131D | -6.175 | 5.89 | -2.032 | 7.601 | 9.263 | 4.423 | 24 | 30.448 | 73.823 | 73.271 |
| CODA1140 | R101F_L131N_S172D | 52.592 | 61.248 | 71.678 | 71.881 | 73.807 | 75.649 | 76 | 76.329 | 76.168 | 74.076 |
| CODA1157 | Q139E_S172D | 68.362 | 69.495 | 75.078 | 74.193 | 77.481 | 77.205 | 77 | 77.042 | 78.2 | 76.244 |
| CODA1173 | S172D_Y210W | 16.321 | 30.264 | 61.978 | 65.343 | 73.006 | 74.903 | 76 | 76.759 | 76.928 | 76.619 |

**Table 13: YFP fluorescence quenching using TC-6683**

| Name | Amino Acid Mutation(s) in SEQ ID NO: 33 | 30nM TC-6683 | 300nM TC-6683 | 3uM TC-6683 | 30uM TC-6683 | 100uM TC-6683 |
|---|---|---|---|---|---|---|
| CODA71 | - | -14.43 | -13.08 | -13.71 | -14.02 | -8.77 |
| CODA282 | Y115D | 0.365 | -6.865 | -4.927 | -11.259 | -11.894 |
| CODA325 | L131G | -21.27 | -18.33 | -7.03 | 56.84 | 64.51 |
| CODA326 | L131A | -16.28 | -11.73 | -2.38 | -9.77 | 0.97 |
| CODA330 | L131F | -22.26 | -18.89 | -9.45 | -9.23 | -10.75 |
| CODA333 | L131S | -23.88 | -21.46 | -21.07 | -16.15 | -18.96 |
| CODA334 | L131Q | -17.99 | -23.26 | -18.84 | -16.92 | -15.59 |
| CODA335 | L131T | -13.84 | -20.66 | -17.86 | -18.91 | -16.88 |
| CODA339 | L131D | -18.32 | -17.25 | -10 | -8.51 | -9.77 |
| CODA340 | L131E | 30.14 | 33.02 | 41.68 | 35.75 | 24.32 |
| CODA342 | L131R | -16.26 | -22.45 | -13.48 | -12.68 | -10.33 |
| CODA337 | L131N | -11.51 | -13.17 | -5.46 | 8.53 | 14.41 |
| CODA945 | Q139E | -26.47 | -18.62 | -13.39 | -16.6 | -12.89 |
| CODA377 | S172D | -20.23 | -13.94 | -14.17 | 1.86 | 43.61 |
| CODA534 | L131S_S172D | -22.464 | -23.976 | -24.897 | -20.474 | -22.253 |
| CODA535 | L131T_S172D | -29.362 | -28.954 | -23.708 | -23.431 | -18.324 |
| CODA536 | L131D_S172D | -22.285 | -23.926 | -20.514 | -3.078 | 7.37 |
| CODA807 | Y115D_L131E | -26.801 | -25.363 | -23.651 | -26.235 | -24.182 |
| CODA952 | Y140I | -27.7 | -26.87 | -31.2 | -33.12 | -24.67 |
| CODA965 | Y140C | -8.21 | -16.37 | -19.48 | -24.32 | -24.99 |
| CODA1025 | R101F_L131G | -1.645 | 3.043 | 0.082 | 2.449 | 10.437 |
| CODA1027 | R101F_L131D | 1.189 | 0.939 | 1.327 | -0.845 | -3.136 |
| CODA1039 | Y115E_Y210W | -1.608 | -3.491 | -6.153 | -9.263 | -9.19 |
| CODA1045 | R101W_Y210V | 1.735 | 0.642 | 2.403 | 6.006 | -4.277 |
| CODA1047 | R101F_Y210V | -24.195 | -24.234 | -21.291 | -16.363 | -24.9 |
| CODA1048 | R101F_Y210F | -29.055 | -33.935 | -24.613 | -21.752 | -22.815 |
| CODA1054 | R101M_L131F | -22.996 | -27.055 | -18.611 | -13.879 | -21.521 |
| CODA1055 | R101W_Y115E_Y210W | -30.056 | -30.801 | -25.558 | -18.295 | -17.554 |
| CODA1056 | R101F_Y115E_Y210W | -19.889 | -22.443 | -12.623 | -10.728 | -10.959 |

**Table 14: YFP fluorescence quenching using TC-5619/Bradanicline**

| Name | Amino Acid Mutation(s) in SEQ ID NO: 33 | 1 nM brd | 3 nM brd | 10 nM brd | 30 nM brd | 100 nM brd | 300 nM brd | 1 uM brd |
|---|---|---|---|---|---|---|---|---|
| CODA71 | - | 9.41 | 44.38 | 76.61 | 70.23 | 78.62 | 75.51 | |
| CODA75 | SEQ ID NO: 29 | -13.39 | -15.78 | -13.47 | -16.41 | | -17.56 | |
| CODA217 | W77F | | -12.1 | | -14.72 | | -6.3 | |
| CODA109 | S170T | | 70.58 | | 80.71 | | 80.66 | |
| CODA236 | R101F | | 49.33 | | 77.38 | | 77.51 | |
| CODA237 | R101M | | -14.87 | | 69.93 | | 76.57 | |
| CODA238 | R101W | | 74.7 | | 76.78 | | 77.01 | |
| CODA282 | Y115D | -0.54 | -7.22 | -2.82 | -16.67 | -0.02 | -12.37 | 31.23 |
| CODA283 | Y115E_N379K | 0.59 | -6.41 | -1.58 | -14.24 | 33.36 | 43.81 | 75.39 |
| CODA325 | L131G | | 68.45 | | 76.95 | | 77.1 | |
| CODA326 | L131A | | 72.24 | | 77.4 | | 78.02 | |
| CODA330 | L131F | -12.4 | 25.12 | 72.19 | 76.35 | 78.94 | 77.06 | 78.61 |
| CODA333 | L131S | -0.85 | -2.05 | -2.8 | -2.07 | 30.34 | 50.56 | 72.42 |
| CODA334 | L131Q | | 41.92 | | 63.65 | | 64.85 | |
| CODA335 | L131T | -2.39 | -4.08 | -3.3 | -9.69 | -1.05 | 10.53 | 34.72 |
| CODA339 | L131D | 4.12 | 19.15 | 68.98 | 77.29 | 81.41 | 75.79 | 82.26 |
| CODA340 | L131E | | 68.57 | | 76.85 | | 78.49 | |
| CODA342 | L131R | -7.83 | -12.89 | -10.47 | 59.48 | 70.19 | 75.11 | 77.42 |
| CODA337 | L131N | | 71.02 | | 78.43 | | 77.12 | |
| CODA945 | Q139E | | 14.15 | | 78.18 | | 76.8 | |
| CODA377 | S172D | 0.77 | -4.59 | 0.06 | -10.48 | 0.25 | -10.61 | 3.82 |
| CODA405 | Y210V | | 9.92 | | 4.37 | | 59 | |
| CODA407 | Y210F | | -0.53 | | 8.71 | | 76.26 | |
| CODA409 | Y210W | -13.45 | -0.64 | 37.3 | 76.36 | 72.72 | 75.47 | 78.73 |
| CODA534 | L131S_S172D | -1.13 | -3.83 | -3.78 | | -2.03 | | 1.68 |
| CODA536 | L131D_S172D | -1.24 | 0.25 | -2.57 | 0.78 | 4.51 | 30.97 | 44.41 |
| CODA805 | Y115D_S170T | -9.78 | -12.06 | -9.02 | -16.3 | -7.44 | -10.93 | -4.55 |
| CODA806 | Y115D_L131Q | 0.92 | -4.65 | 0.38 | -8.96 | 12.18 | -0.3 | 45.47 |
| CODA807 | Y115D_L131E | -1.52 | -5.35 | -3.49 | -2.16 | 4.18 | 70.2 | 43.11 |
| CODA952 | Y140I | | -2.45 | | 66.97 | | 78.48 | |
| CODA965 | Y140C | | -18.49 | | 22.66 | | 38.02 | |
| CODA1025 | R101F_L131G | -10.61 | 2.63 | 17.71 | 68.48 | | 75.12 | |
| CODA1027 | R101F_L131D | -10.07 | -0.8 | 1.74 | 53.95 | | 76.74 | |
| CODA1039 | Y115E_Y210W | -5.55 | 6.78 | -2.7 | 34.43 | | 70.05 | |
| CODA1045 | R101W_Y210V | -1.431 | -14.993 | -5.52 | 15.49 | 72.105 | 71.411 | |

| Name | Amino Acid Mutation(s) in SEQ ID NO: 33 | 1 nM brd | 3 nM brd | 10 nM brd | 30 nM brd | 100 nM brd | 300 nM brd | 1 uM brd |
|---|---|---|---|---|---|---|---|---|
| CODA1047 | R101F_Y210V | -5.548 | -9.432 | -3.068 | 2.368 | 47.529 | 74.413 | |
| CODA1048 | R101F_Y210F | -2.084 | -9.098 | -2.587 | 55.818 | 76.957 | 74.268 | |
| CODA1053 | R101M_L131A | 0.008 | 43.21 | 77.672 | 76.277 | 76.856 | 75.32 | |
| CODA1054 | R101M_L131F | 6.609 | -7.635 | 4.459 | 69.782 | 77.633 | 72.933 | |
| CODA1055 | R101W_Y115E_Y210W | -8.113 | -11.136 | 38.755 | 39.111 | 61.378 | 52.398 | |
| CODA1056 | R101F_Y115E_Y210W | -7.894 | -11.812 | 36.211 | 40.624 | 65.869 | 59.661 | |

**Table 15: YFP fluorescence quenching using CNL002**

| Name | Amino Acid Mutation(s) in SEQ ID NO: 33 | 1nM CNL002 | 10nM CNL002 | 50nM CNL002 | 100 nM CNL002 | 1 uM CNL002 | 30 uM CNL002 |
|---|---|---|---|---|---|---|---|
| CODA71 | - | | | | 49.61 | 74.15 | 75.21 |
| CODA75 | SEQ ID NO: 29 | | | | -7.94 | -9.81 | -15.27 |
| CODA217 | W77F | | | | 67.81 | 69.42 | 73.64 |
| CODA109 | S170T | | | | 73.48 | 70.03 | |
| CODA236 | R101F | | | | -9.56 | 56.9 | 70.28 |
| CODA237 | R101M | | | | -8.68 | -2.24 | 66.99 |
| CODA238 | R101W | | | | 2.95 | 67.93 | 73.02 |
| CODA282 | Y115D | | | | -9.7 | -8.37 | 3.4 |
| CODA283 | Y115E_N379K | | | | -5.81 | 9.69 | -12.85 |
| CODA325 | L131G | | | | 45.62 | 74.72 | 77.4 |
| CODA326 | L131A | | | | 27.88 | 73.79 | 75.89 |
| CODA330 | L131F | | | | -3.76 | 68.51 | 77.77 |
| CODA333 | L131S | | | | -15.44 | 15.21 | |
| CODA334 | L131Q | | | | 49.9 | 80.72 | 80.97 |
| CODA335 | L131T | | | | -14.53 | 6.45 | 57.43 |
| CODA339 | L131D | | | | 12.83 | 77.73 | 80.58 |
| CODA340 | L131E | | | | 49.97 | 67.8 | 64.58 |
| CODA342 | L131R | | | | -17.2 | 8.12 | 57.24 |
| CODA337 | L131N | | | | 76.37 | 80.18 | 80.93 |
| CODA945 | Q139E | | | | 60.5 | 75.41 | 77.46 |
| CODA377 | S172D | | | | 59.35 | 79.26 | 79.96 |
| CODA405 | Y210V | | | | -14.3 | -7.56 | -2.02 |
| CODA407 | Y210F | | | | -12.47 | -3.93 | 80.97 |
| CODA409 | Y210W | | | | -14.92 | -0.85 | 29.62 |
| CODA534 | L131S_S172D | | | | 14.26 | 45.55 | 64.93 |
| CODA535 | L131T_S172D | | | | 1.23 | 35.77 | 68.29 |

| Name | Amino Acid Mutation(s) in SEQ ID NO: 33 | 1nM CNL002 | 10nM CNL002 | 50nM CNL002 | 100 nM CNL002 | 1 uM CNL002 | 30 uM CNL002 |
|---|---|---|---|---|---|---|---|
| CODA536 | L131D_S172D | | | | 71.72 | 77.26 | 77.5 |
| CODA805 | Y115D_S170T | | | | -18.17 | -15.2 | -14 |
| CODA806 | Y115D_L131Q | | | | -10.03 | -3.33 | 6.96 |
| CODA807 | Y115D_L131E | | | | -5.71 | 13.03 | 25.2 |
| CODA952 | Y140I | | | | 32.58 | 69.08 | 73.28 |
| CODA965 | Y140C | | | | 43.24 | 42.72 | 51.43 |
| CODA1025 | R101F_L131G | | | | 12.48 | 46.04 | 39.82 |
| CODA1027 | R101F_L131D | | | | 7.75 | 61.97 | 65.77 |
| CODA1039 | Y115E_Y210W | | | | 21.85 | 47.37 | 59.97 |
| CODA1138 | W77F_R101F_L131D | -2.209 | 43.044 | 73.418 | 81.66 | 81.69 | |
| CODA1140 | R101F_L131N_S172D | 0.337 | 59.894 | 76.501 | 79.03 | 82.85 | |
| CODA1157 | Q139E_S172D | | | | 80.62 | 81.32 | |
| CODA1173 | S172D_Y210W | | | | 70.15 | 77.33 | |

**Table 16: YFP fluorescence quenching using ABT-126**

| Name | Amino Acid Mutation(s) in SEQ ID NO: 33 | 50 nM abt | 100 nM abt | 300 nM abt | 500 nM abt | 1 uM abt | 3 uM abt | 10 uM abt | 30 uM abt | 50 uM abt | 100 uM abt |
|---|---|---|---|---|---|---|---|---|---|---|---|
| CODA71 | - | -1.98 | 24.27 | 56.8 | 76.28 | 73.16 | 70.98 | 72.62 | 77.49 | 77.96 | 77.81 |
| CODA75 | SEQ ID NO: 29 | -17.07 | -16.76 | -16.76 | -14.68 | -16 | -14.11 | -13.98 | -21.05 | -21.35 | -24.12 |
| CODA217 | W77F | 37.81 | 57.31 | 12.61 | | 66.05 | | | | | |
| CODA109 | S170T | 55.83 | 68.89 | 72.52 | | 70.96 | | | | | |
| CODA236 | R101F | -18.69 | -23.01 | -20.92 | | 4.99 | | | | | |
| CODA237 | R101M | -24.34 | -20.22 | -20.57 | | -19.55 | | | | | |
| CODA238 | R101W | -14.97 | -14.66 | -16.35 | | 50.02 | | | | | |
| CODA282 | Y115D | -21.22 | -19.7 | -15.55 | | -14.31 | | | | | |
| CODA283 | Y115E_N379K | -22.65 | -20.55 | -12.93 | | 1.71 | | | | | |
| CODA325 | L131G | -17.39 | 6.89 | 38.65 | | 45.82 | | | | | |
| CODA326 | L131A | -21.91 | -0.5 | 34.86 | | 56.66 | | | | | |
| CODA330 | L131F | -16.22 | -11.89 | 39.23 | | 66.5 | | | | | |
| CODA333 | L131S | -20.99 | -16.21 | -13.34 | | 4.96 | | | | | |
| CODA334 | L131Q | 23.65 | 61.83 | 76.44 | | 77.68 | | | | | |
| CODA335 | L131T | -18 | -16.39 | -15.05 | | -5.56 | | | | | |
| CODA339 | L131D | -16.65 | -12.3 | 18.5 | | 59.09 | | | | | |
| CODA340 | L131E | 2.2 | 25.39 | 36.48 | | 50.76 | | | | | |
| CODA342 | L131R | -18.14 | -18.14 | -0.81 | 23.29 | 45.76 | 53.03 | 64.08 | 71.98 | 73.98 | 73.51 |
| CODA337 | L131N | -11.64 | 34.79 | 60.08 | | 65.99 | | | | | |
| CODA945 | Q139E | -8.27 | 4.76 | 30.88 | | 60.66 | | | | | |
| CODA377 | S172D | -1.53 | 18.18 | 59.15 | | 72.28 | | | | | |

| Name | Amino Acid Mutation(s) in SEQ ID NO: 33 | 50 nM abt | 100 nM abt | 300 nM abt | 500 nM abt | 1 uM abt | 3 uM abt | 10 uM abt | 30 uM abt | 50 uM abt | 100 uM abt |
|---|---|---|---|---|---|---|---|---|---|---|---|
| CODA405 | Y210V | -2.9 | -2.06 | -0.19 | | -3.64 | | | | | |
| CODA407 | Y210F | -13.07 | 0.1 | 5.97 | | 50.23 | | | | | |
| CODA409 | Y210W | 8.23 | 55.53 | 74.82 | 77.39 | 77.77 | 69.33 | 74.79 | 75.61 | 75.72 | 78.52 |
| CODA534 | L131S_S172D | -11.96 | -10.72 | 11.65 | | -3.06 | | | | | |
| CODA535 | L131T_S172D | -11.09 | -10.37 | -4 | | 4.05 | | | | | |
| CODA536 | L131D_S172D | -3.19 | 7.58 | -1.65 | | 31.29 | | | | | |
| CODA805 | Y115D_S170T | -11.83 | -11.5 | -8.01 | | -9.34 | | | | | |
| CODA806 | Y115D_L131Q | -1.81 | -2.61 | -3.78 | | -2.6 | | | | | |
| CODA807 | Y115D_L131E | -1.68 | -2.25 | -2.37 | | 3.68 | | | | | |
| CODA952 | Y140I | -12.34 | -11.57 | -5.21 | | 3.93 | | | | | |
| CODA965 | Y140C | -10.75 | -4.11 | 18.93 | | 52.64 | | | | | |
| CODA1025 | R101F_L131G | -11.53 | -7.61 | 6.55 | | 25.08 | | | | | |
| CODA1027 | R101F_L131D | -11.89 | -16.07 | -11.21 | | -9.38 | | | | | |
| CODA1039 | Y115E_Y210W | -17.32 | -13.93 | 9.9 | 36.97 | 48.37 | 50.5 | 58.13 | 64.1 | 66.67 | 68.63 |
| CODA1045 | R101W_Y210V | -24.16 | -24.7 | -16.09 | | -11.61 | | | | | |
| CODA1047 | R101F_Y210V | -14.1 | -11.72 | -12.98 | | -15.09 | | | | | |
| CODA1048 | R101F_Y210F | -23.28 | -21.78 | -19.35 | | -16.33 | | | | | |
| CODA1053 | R101M_L131A | -27.01 | -18.76 | -17.8 | | -6.55 | | | | | |
| CODA1054 | R101M_L131F | -19.54 | -18.28 | -16.87 | | -11.77 | | | | | |
| CODA1055 | R101W_Y115E_Y210W | -21.36 | -22.28 | -14.63 | | -7.34 | | | | | |
| CODA1056 | R101F_Y115E_Y210W | -20.96 | -21.95 | -10.96 | | 10.79 | | | | | |
| CODA1138 | W77F_R101F_L131D | -11.71 | -5.13 | 15.09 | | 56.63 | | 75.93 | | | |
| CODA1140 | R101F_L131N_S172D | -7.87 | -5.35 | -3.67 | | 3.64 | | 5.91 | | | |

| Name | Amino Acid Mutation(s) in SEQ ID NO: 33 | 50 nM abt | 100 nM abt | 300 nM abt | 500 nM abt | 1 uM abt | 3 uM abt | 10 uM abt | 30 uM abt | 50 uM abt | 100 uM abt |
|---|---|---|---|---|---|---|---|---|---|---|---|
| CODA1157 | Q139E_S172D | -26.77 | -24.64 | -10.64 | | 19.57 | | 55.64 | | | |
| CODA1173 | S172D_Y210W | 5.15 | 25.08 | 66.86 | | 66.94 | | 78.67 | | | |

**Table 17: YFP fluorescence quenching using AZD-0328**

| Name | Amino Acid Mutation(s) in SEQ ID NO: 33 | 100 nM azd0328 | 300 nM azd0328 | 1 uM azd0328 | 10 uM azd0328 | 30 uM azd0328 |
|---|---|---|---|---|---|---|
| CODA71 | - | 73.59 | 78.09 | | | 79.05 |
| CODA75 | SEQ ID NO: 29 | -8.83 | -11.15 | | | -11.92 |
| CODA217 | W77F | -7.57 | 7.08 | | | 81.59 |
| CODA109 | S170T | 84.01 | 87.08 | | | 87.27 |
| CODA236 | R101F | -9.78 | 49.64 | | | 81.14 |
| CODA237 | R101M | -17.84 | -11.98 | | | 81.2 |
| CODA238 | R101W | 22.94 | 75.38 | | | 81.8 |
| CODA282 | Y115D | 3.19 | 4.79 | | | 76.78 |
| CODA283 | Y115E_N379K | -2.1 | 38.2 | | | 79.44 |
| CODA325 | L131G | 79.75 | 80.62 | | | 80.19 |
| CODA326 | L131A | 80.39 | 82.07 | | | 82.23 |
| CODA330 | L131F | 27.56 | 73.35 | | | 77.14 |
| CODA333 | L131S | 38.49 | 67.13 | | | 80.61 |
| CODA334 | L131Q | 80.55 | 83.15 | | | 82.53 |
| CODA335 | L131T | 11.33 | 44.15 | | | 80.96 |
| CODA339 | L131D | 62.87 | 77.03 | | | 78.63 |
| CODA340 | L131E | 82.48 | 82.04 | | | 81.8 |
| CODA342 | L131R | 1.79 | 66.58 | | | 80.55 |
| CODA337 | L131N | 82.1 | 82.52 | | | 82.32 |
| CODA945 | Q139E | -5.79 | 58.47 | | | 66.57 |
| CODA377 | S172D | 65.04 | 81.73 | | | 82.3 |
| CODA405 | Y210V | -8.13 | -3.35 | | | -2.86 |
| CODA407 | Y210F | 0.85 | 1.25 | | | 82.08 |
| CODA409 | Y210W | -6.57 | -4.84 | | | 81.64 |
| CODA534 | L131S_S172D | -9.32 | 4.94 | | | 60.61 |
| CODA535 | L131T_S172D | -7.02 | -0.48 | | | 70.16 |
| CODA536 | L131D_S172D | 51.44 | 77.12 | | | 79.26 |
| CODA805 | Y115D_S170T | -0.71 | -1.73 | | | 47.39 |
| CODA806 | Y115D_L131Q | 12.15 | 30.92 | | | 68.65 |
| CODA807 | Y115D_L131E | 24.49 | 45.34 | | | 71.21 |
| CODA952 | Y140I | -12.96 | -13.21 | | | 68.5 |
| CODA965 | Y140C | -16.69 | 1.3 | | | 21.78 |
| CODA1025 | R101F_L131G | 6.26 | 37.98 | | | 79.31 |
| CODA1027 | R101F_L131D | -4.76 | -7.52 | | | 74.9 |
| CODA1039 | Y115E_Y210W | -10.45 | -13 | | | 30.76 |

**Table 18: YFP fluorescence quenching using Facinicline**

| Name | Amino Acid Mutation(s) in SEQ ID NO: 33 | 30 nM fac | 50 nM fac | 100 nM fac | 300 nM fac | 1 uM fac | 10 uM fac | 30 uM fac |
|---|---|---|---|---|---|---|---|---|
| CODA71 | - | 53.42 | | 83.6 | 77.44 | | | 79.62 |
| CODA75 | SEQ ID NO: 29 | -11.68 | | 4.35 | -10.55 | | | -5.25 |
| CODA217 | W77F | -10.14 | | | 68.29 | | | 87.35 |
| CODA109 | S170T | 76.45 | | 82.45 | 71.09 | | | 69.41 |
| CODA236 | R101F | -28.54 | | | 72.98 | | | 87.6 |
| CODA237 | R101M | -24.14 | | | -14.38 | | | 88.78 |
| CODA238 | R101W | -19.61 | | | 76.54 | | | 87.94 |
| CODA282 | Y115D | 10.17 | | 24.77 | 51.04 | | 79.76 | 81.7 |
| CODA283 | Y115E_N379K | 6.39 | | | 78.36 | | 81.93 | 78.63 |
| CODA325 | L131G | 75.75 | | | 78.01 | | | 76.61 |
| CODA326 | L131A | 69.94 | | | 80.07 | | | 78.32 |
| CODA330 | L131F | 2.9 | | | 74.96 | | 81.97 | 78.31 |
| CODA333 | L131S | 6.27 | | | 69.8 | | 80.07 | 81.35 |
| CODA334 | L131Q | 83.07 | | 85.36 | 84.19 | | | 82.75 |
| CODA335 | L131T | 1.24 | | | 46.92 | | 79.06 | 81.06 |
| CODA339 | L131D | 12.67 | | | 80.4 | | 82.18 | 82.14 |
| CODA340 | L131E | 82.51 | | 83.6 | 82.92 | | | 82.68 |
| CODA342 | L131R | -6.2 | | | 76.75 | | 82.16 | 81.71 |
| CODA337 | L131N | 82.25 | | | 83.59 | | | 82.82 |
| CODA945 | Q139E | -7.16 | | | 71.17 | | | 72.14 |
| CODA377 | S172D | 4.93 | | | 84.13 | | 66.96 | 85.29 |
| CODA405 | Y210V | -26.25 | | | -23.52 | | | 0.83 |
| CODA407 | Y210F | -21.94 | | | -22.46 | | | 82.63 |
| CODA409 | Y210W | -10.93 | | | -5.82 | | 60.97 | 57.77 |
| CODA534 | L131S_S172D | -23.44 | | | 2.75 | | 69.71 | 61.44 |
| CODA535 | L131T_S172D | -21.36 | | | 4.17 | | | 65.94 |
| CODA536 | L131D_S172D | 24.15 | | | 80.17 | | 81.34 | 79.9 |
| CODA805 | Y115D_S170T | 38.08 | | -14.37 | 19.41 | | 67.33 | 79.67 |
| CODA806 | Y115D_L131Q | 41.32 | | 59.51 | 74.86 | | 78.53 | 84.45 |
| CODA807 | Y115D_L131E | 45.76 | | 63.92 | 79.43 | | 80.1 | 84.91 |
| CODA952 | Y140I | -8.83 | | | 51.6 | | | 71.01 |
| CODA965 | Y140C | -12.29 | | | 20.11 | | | 37.62 |

| Name | Amino Acid Mutation(s) in SEQ ID NO: 33 | 30 nM fac | 50 nM fac | 100 nM fac | 300 nM fac | 1 uM fac | 10 uM fac | 30 uM fac |
|---|---|---|---|---|---|---|---|---|
| CODA1025 | R101F_L131G | -8.99 | | | 10.21 | | | 78.24 |
| CODA1027 | R101F_L131D | -10.05 | | | -12.54 | | | 70.96 |
| CODA1039 | Y115E_Y210W | -13.46 | | | 5.43 | | | 49.06 |

**Table 19: YFP fluorescence quenching using TC-6987**

| Name | Amino Acid Mutation(s) in SEQ ID NO: 33 | 1 uM tc6987 | 3 uM tc6987 | 5 uM tc6987 | 10 uM tc6987 | 30 uM tc6987 | 50 uM tc6987 | 100 uM tc6987 | 300 uM tc6987 | 500 uM tc6987 |
|---|---|---|---|---|---|---|---|---|---|---|
| CODA71 | - | 31.56 | 31.82 | 30.75 | 56.68 | 51.46 | 61.26 | 43.21 | 7.79 | 51.58 |
| CODA75 | SEQ ID NO: 29 | -11.79 | | | -14.31 | | | | | -19.65 |
| CODA217 | W77F | -10.35 | | | -13.93 | | | | | -7.54 |
| CODA109 | S170T | 71.45 | | | 71.04 | | | | | 66.2 |
| CODA236 | R101F | 45.34 | | | 70.04 | | | | | 75.86 |
| CODA237 | R101M | -21.62 | | | 21.52 | | | | | 60.33 |
| CODA238 | R101W | 72.47 | | | 72.04 | | | | | 76.56 |
| CODA282 | Y115D | 13.01 | | | 14.73 | | | | | 14.29 |
| CODA283 | Y115E_N379K | -9.54 | | | -7.06 | | | | | -15.15 |
| CODA325 | L131G | 64.59 | | | 76.28 | | | | | 70.95 |
| CODA326 | L131A | 73.37 | | | 78.18 | | | | | 75.42 |
| CODA330 | L131F | 53.37 | | | 74.23 | | | | | 72.25 |
| CODA333 | L131S | -3.87 | -20.2 | -15.25 | 4.92 | 14.75 | 21.33 | 29.7 | 20.79 | 44.37 |
| CODA334 | L131Q | 74.1 | | | 81.7 | | | | | 80.18 |
| CODA335 | L131T | -0.14 | -8.32 | -2.13 | 0.13 | -6.86 | -9.5 | -10.98 | -12.16 | -2.41 |
| CODA339 | L131D | 50 | 80.39 | 79.93 | 80.99 | 83.11 | 83.18 | 82.16 | 64.58 | 76.77 |
| CODA340 | L131E | 80.13 | | | 81.29 | | | | | 79.6 |
| CODA342 | L131R | 2.51 | | | 46.22 | | | | | 68.33 |
| CODA337 | L131N | 79.06 | | | 82.36 | | | | | 80.19 |
| CODA945 | Q139E | -0.97 | | | 21.53 | | | | | 7.39 |
| CODA377 | S172D | -9.74 | -14.04 | -0.95 | -7.41 | -16.12 | -12.79 | -11.77 | -19.68 | -17.02 |

| Name | Amino Acid Mutation(s) in SEQ ID NO: 33 | 1 uM tc6987 | 3 uM tc6987 | 5 uM tc6987 | 10 uM tc6987 | 30 uM tc6987 | 50 uM tc6987 | 100 uM tc6987 | 300 uM tc6987 | 500 uM tc6987 |
|---|---|---|---|---|---|---|---|---|---|---|
| CODA405 | Y210V | -22.63 | | | -23.03 | | | | | -40.88 |
| CODA407 | Y210F | -15.22 | | | -19.89 | | | | | -33.32 |
| CODA409 | Y210W | 62.82 | | | 78.66 | | | | | 70.35 |
| CODA534 | L131S_S172D | -20.99 | | | -27.62 | | | | | -24.22 |
| CODA535 | L131T_S172D | -21.8 | | | -27.08 | | | | | -21.56 |
| CODA536 | L131D_S172D | -18.24 | | | -23.06 | | | | | -19.3 |
| CODA805 | Y115D_S170T | 31.9 | | | 25.54 | | | | | 21.13 |
| CODA806 | Y115D_L131Q | 38.83 | | | 31.59 | | | | | 29.83 |
| CODA807 | Y115D_L131E | 36.07 | | | 31.35 | | | | | 28.18 |
| CODA952 | Y140I | 6.03 | | | 2.25 | | | | | -0.24 |
| CODA965 | Y140C | 2.73 | | | 30.37 | | | | | 33.07 |
| CODA1025 | R101F_L131G | -13.32 | | | 43.71 | | | | | 53.98 |
| CODA1027 | R101F_L131D | 0.88 | | | 56.27 | | | | | 71.3 |
| CODA1039 | Y115E_Y210W | 7.26 | | | 11 | | | | | 7.97 |

**Table 20: YFP fluorescence quenching using Varenicline**

| Name | Amino Acid Mutation(s) in SEQ ID NO: 33 | 3nM var | 3nM var | 30nM var | 30nM var | 300nM var | 300nM var | 500nM var | 500nM var | 1uM var | 1uM var |
|---|---|---|---|---|---|---|---|---|---|---|---|
| CODA71 | - | -2.36 | -3.522 | -4.255 | -3.636 | 46.195 | 45.522 | 69.253 | 71.705 | 79.254 | 79.244 |
| CODA75 | SEQ ID NO: 29 | 4.161 | -6.216 | -5.173 | -6.593 | -5.733 | -7.312 | -10.35 | -7.586 | -5.637 | -3.757 |
| CODA217 | W77F | 5.662 | 7.57 | 13.968 | 19.079 | 16.183 | 18.025 | 23.366 | 19.206 | 34.69 | 30.504 |
| CODA109 | S170T | -20.96 | -19.953 | -16.094 | -21.502 | 64.373 | 71.198 | 71.892 | 73.53 | 75.461 | 78.578 |
| CODA236 | R101F | 19.949 | 22.334 | 15.764 | 16.213 | 23.135 | 22.987 | 35.048 | 34.188 | 71.031 | 71.005 |
| CODA237 | R101M | 18.429 | 16.83 | 23.07 | 15.868 | 18.819 | 19.155 | 19.117 | 22.593 | 24.525 | 23.383 |
| CODA238 | R101W | 17.896 | 12.564 | 16.263 | 22.117 | 77.492 | 79.056 | 80.186 | 80.065 | 81.263 | 80.702 |
| CODA282 | Y115D | 2.111 | -12.265 | -4.433 | -6.153 | -0.861 | -1.308 | 0.733 | -4.633 | -4.512 | 0.02 |
| CODA283 | Y115E_N379K | | | | | | | | | | |
| CODA325 | L131G | -12.241 | -9.415 | 75.334 | 72.278 | 78.305 | 77.83 | 75.936 | 76.788 | 77.805 | 77.352 |
| CODA326 | L131A | -5.939 | -2.294 | 68.663 | 70.718 | 79.88 | 79.742 | 79.311 | 80.029 | 80.952 | 79.329 |
| CODA330 | L131F | -3.016 | -1.452 | -0.193 | -1.853 | 6.915 | 6.513 | 27.593 | 29.863 | 67.499 | 65.57 |
| CODA333 | L131S | -4.782 | 1.048 | 2.084 | 5.022 | 12.162 | 12.979 | 29.238 | 25.614 | 43.863 | 39.346 |
| CODA334 | L131Q | -5.192 | -4.327 | -0.688 | 0.672 | 75.189 | 74.923 | 79.048 | 80.337 | 79.766 | 76.836 |
| CODA335 | L131T | 0.398 | 3.947 | 5.31 | 7.666 | 4.327 | 3.275 | 1.835 | 4.863 | 9.471 | 7.505 |
| CODA339 | L131D | -0.902 | -7.62 | -6.009 | -3.447 | 49.328 | 47.685 | 66.7 | 64.798 | 70.85 | 69.038 |
| CODA340 | L131E | -4.061 | -0.568 | 62.964 | 63.631 | 74.912 | 75.084 | 76.346 | 75.876 | 75.805 | 74.856 |
| CODA342 | L131R | 0.271 | -3.613 | -4.935 | -4.363 | 0.282 | 2.32 | 23.777 | 18.376 | 66.17 | 66.088 |
| CODA337 | L131N | 3.093 | 12.327 | 77.097 | 77.653 | 79.446 | 79.998 | 80.691 | 80.309 | 80.258 | 79.509 |
| CODA945 | Q139E | 8.207 | 7.835 | 9.509 | 10.204 | 9.818 | 12.539 | 16.762 | 15.21 | 48.292 | 54.096 |
| CODA377 | S172D | -3.433 | -5.737 | -5.319 | -6.175 | -4.135 | -8.614 | -6.062 | -6.125 | -14.134 | -15.373 |
| CODA405 | Y210V | 7.317 | 7.643 | 9.457 | 9.134 | 15.503 | 11.954 | 13.427 | 10.784 | 9.032 | 11.591 |

| Name | Amino Acid Mutation(s) in SEQ ID NO: 33 | 3nM var | 3nM var | 30nM var | 30nM var | 300nM var | 300nM var | 500nM var | 500nM var | 1uM var | 1uM var |
|---|---|---|---|---|---|---|---|---|---|---|---|
| CODA407 | Y210F | 2.441 | 7.326 | 1.275 | 12.96 | 13.119 | 2.355 | 11.539 | 6.571 | 8.397 | 7.309 |
| CODA409 | Y210W | 7.776 | -2.964 | -5.568 | 3.024 | 2.815 | 3.06 | 1.766 | 2.819 | 1.844 | -4.059 |
| CODA952 | Y140I | -22.522 | -18.173 | -15.306 | -12.832 | -14.671 | -17.016 | -16.713 | -18.893 | -15.847 | -16.236 |
| CODA965 | Y140C | -17.716 | -23.019 | -14.993 | -12.182 | -11.731 | -10.13 | -12.518 | -15.043 | -13.385 | -17.022 |
| CODA1025 | R101F_L131G | 48.074 | 60.978 | 70.023 | 69.433 | 78.989 | 79.21 | 79.212 | 79.771 | 79.538 | 80.577 |
| CODA1027 | R101F_L131D | -3.367 | -0.431 | -0.761 | 2.989 | 2.966 | 10.211 | 15.278 | 20.84 | 37.104 | 42.439 |
| CODA1039 | Y115E_Y210W | -10.926 | -7.395 | -5.661 | -1.244 | -4.555 | 1.081 | 9.172 | 12.561 | 7.139 | 12.664 |
| CODA1045 | R101W_Y210V | 6.745 | 8.397 | 14.1 | 19.095 | 16.83 | 21.507 | 21.586 | 23.131 | 31.591 | 26.479 |
| CODA1047 | R101F_Y210V | -3.035 | -0.786 | -5.181 | 0.669 | -2.748 | -6.708 | -9.123 | -9.869 | -6.702 | -7.26 |
| CODA1048 | R101F_Y210F | -17.119 | -13.234 | -7.747 | 4.193 | 4.149 | 1.512 | -1.572 | 3.71 | 8.414 | 4.091 |
| CODA1053 | R101M_L131A | 74.835 | 76.119 | 77.608 | 79.015 | 80.71 | 79.002 | 78.709 | 78.884 | 79.688 | 80 |
| CODA1054 | R101M_L131F | 34.923 | 28.944 | 42.214 | 43.015 | 47.059 | 52.768 | 59.542 | 62.641 | 71.449 | 69.145 |
| CODA1055 | R101W_Y115E_Y210W | 0.468 | -10.015 | -15.532 | -14.462 | -17.027 | -15.59 | -13.343 | -10.702 | -6.604 | -7.612 |
| CODA1056 | R101F_Y115E_Y210W | | | | | | | | | | |
| CODA1138 | W77F_R101F_L131D | 3.893 | 1.107 | 0.972 | 0.872 | 2.282 | -0.288 | 3.637 | -1.804 | -1.84 | 6.36 |
| CODA1140 | R101F_L131N_S172D | -6.169 | -10.445 | -9.679 | -10.878 | -9.572 | -8.873 | -14.656 | -11.304 | -13.996 | -16.128 |
| CODA1157 | Q139E_S172D | -0.225 | -1.954 | 1.269 | 5.972 | 6.84 | 5.943 | 8.717 | 6.41 | 5.635 | 7.068 |
| CODA1173 | S172D_Y210W | 9.211 | 7.193 | 7.821 | 9.078 | 6.933 | 8.36 | 5.501 | 7.04 | 7.174 | 5.185 |

**[0379]** **Table 21A** and **Table 21B** below list the EC50 values from one set of experiments for the indicated mutants, in which the EC50 values to Ach and TC-5619 as determined from the YFP fluorescence plate reader experiments are compared with the EC50 values from the electrophysiology studies as described in Example 3 below. The results in **Table 21A** and **Table 21B** demonstrate that the EC50 values derived from the YFP fluorescence plate reader experiments are in excellent agreement with those derived from high throughput electrophysiology (ephys) studies as described in Example 3 below.

**Table 21A: EC50 values of the engineered receptors to either acetylcholine or TC-5619**

| | Acetylcholine | | TC-5619 | |
|---|---|---|---|---|
| **Name** | **EC50 (plate reader)** | **EC50 (ephys)** | **EC50 (plate reader)** | **EC50 (ephys)** |
| CODA536 | 359 μM | | 757 nM | |
| CODA534 | >3mM | | >1μM | |
| CODA535 | >3mM | | | |
| CODA1027 | >3mM | 3.88 mM | 23.5 nM | 25 nM |
| CODA1025 | 1.28 mM | 993 uM | 14 nM | 17 nM |
| COAD1056 | >3mM | 4.18 mM | 25.1 nM | 42.1 nM |
| CODA1048 | 1.39 mM | 4.78 mM | 21.5 nM | 41.2 nM |
| CODA1047 | >3mM | 14.4 mM | 77 nM | 159 nM |
| CODA1053 | 650 uM | 1.74 mM | 2.62 nM | 3.58 nM |
| CODA1054 | 2.88 mM | 4.74 mM | 18.3 nM | 21.8 nM |
| COAD1055 | >3mM | 3.86 mM | 23.5 nM | 10.6 nM |
| CODA1045 | >3mM | 11.8 mM | 60.5 nM | 70.4 nM |
| CODA807 | 1.74 mM | | 149 nM | |
| CODA806 | 4.61 mM | | 968 nM | |
| CODA805 | >3mM | | >1 μM | |
| CODA1039 | >3mM | 4.82 mM | 30.1 nM | 99.3 nM |
| CODA952 | 575 μM | 1.74 mM | 19.3 nM | |

**Table 21B: EC50 (uM) of the engineered receptors to the indicated ligand (plate reader data using HEK293 cells)**

| Name | Acetyl-choline | ABT-126 | CNL002 | AZD-0328 | TC-5619 | Facinicline | TC-6683 | TC-6987 | Varenicline |
|---|---|---|---|---|---|---|---|---|---|
| CODA967 | 997 | 2.3310 | 22.6037 | 30.1857 | 0.0296 | 24.5615 | 6948.4431 | 1417.9346 | 869.6257 |
| CODA1101 | 649 | 0.0515 | | | | | | 14152.3771 | 853.5533 |
| CODA1167 | 4713 | 0.0630 | 0.0680 | | | | 1578.3355 | 13056.6251 | 1.6545 |
| CODA1168 | 1234 | 0.0421 | 475.7462 | | | | | 5.4513 | 0.8764 |
| CODA1245 | 5056 | 0.0431 | 0.1626 | | | | 7782.5582 | 11693.8156 | 708.3588 |
| CODA1261 | 4763 | 79.0708 | 1.0027 | | | | 681.6739 | 11669.5312 | 452.4647 |
| CODA1458 | 1331 | | 1188.2563 | | | | | | 0.0089 |
| CODA1471 | 1808 | | 1.2352 | | | | | | 417.5071 |
| CODA1500 | 2125 | | 0.0237 | | | | | | 354.8661 |
| CODA1507 | 491 | | 0.0715 | | | | | | 0.2302 |

**Example 3: Characterization of the Engineered Receptors using High Throughput Electrophysiology**

**[0380]** To confirm the $EC_{50}$s determined by plate reader as well as develop a better understanding of maximum current flow, the engineered receptors were subjected to high throughput electrophysiology system, as described below. For HEK293T studies, cDNAs encoding ion channels were cloned into pcDNA3.1 using standard recombination techniques. HEK293T cells from Clontech (Lenti-X™ 293T Cell Line) were cultured in DMEM supplemented with 10% FBS and 1% Pen/Strep to 40-50% confluence using standard cell culture protocols, transfected with ion channel plasmids at a concentration of 18 μg per 15 cm dish using Fugene 6, and grown for an additional 24 hours. Cells were then assayed on an electrophysiological system (IonFluxHT and/or Mercury, Fluxion Biosciences) in which dose-response relationships may be assessed through a microfluidics-based platform for establishing whole cell configurations. Ensemble plates were primed with extracellular buffer (140 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, and 10 mM glucose, pH 7.2 with NaOH, mOsm 310), intracellular buffer (145 mM CsCl, 2 mM CaCl2, 2 mM MgCl2, 10 mM HEPES, and 10 mM EGTA, pH 7.2 with CsOH, mOsm 305), as adapted from Lynagh and Lynch, and test compounds (stocks prepared fresh) diluted in extracellular buffer. Cells were then released from the plate with Accutase, centrifuged, resuspended in extracellular buffer, and loaded into Ensemble plates. Cells were then subjected to a standard protocol for priming, trapping, breaking, and establishing whole cell configuration, with cells held at -60 mV throughout the recording. After recording baseline, progressive doses of test compounds were applied using the IonFlux software to assess dose response relationships. Data were then analyzed off-line using custom Python scripts to convert data to .csv format, re-plot traces, and apply QC measurements to reject unstable recordings (i.e. thresholding based on access resistance and/or standard deviation in baseline, as well as artifact rejection). Peak currents were then calculated, and population data were fit using a 4-parameter logistic equation as described by the Hill Equation. Currents were measured on an automated patch clamp system (Fluxion Biosciences) following 1 second addition of drug and the EC50 values calculated are tabulated below in **Table 22A** and **Table 22B** below and **Table 21A** and **Table 21B** above (in Example 2).

**Table 22A: EC50 from high throughput electrophysiology**

| | | High-throughput Ephys EC50 (uM) | | | | | |
|---|---|---|---|---|---|---|---|
| | | HEK293 Cell Line | | | | | |
| **Name** | **Amino Acid Mutation(s) in SEQ ID NO: 33** | **Acetyl-choline** | **TC-5619** | **CNL002** | **Facini-cline** | ***TC-6987*** | ***AZD-0328*** |
| Wild type nAchRa7 | n/a | 47.9 | | | 2.2 | 3.5 | 1.2 |
| CODA71 | - | 74.1 | 0.04 | 0.132 | 0.24 | | |
| CODA282 | Y115D | 26887 | | | 0.7 | | |
| CODA283 | Y115E | 2295 | | | 0.1 | | |
| CODA333 | L131S | 1759 | | | 0.73 | 26.5 | 0.45 |
| CODA335 | L131T | 6015 | | | 1.76 | | 2.92 |
| CODA339 | L131D | 532 | | | 0.41 | 5.42 | 0.33 |
| CODA377 | S172D | 1037 | | | 0.39 | | 0.3 |
| CODA536 | L131D, S172D | 345 | | _ | 0.132 | | |
| CODA534 | L131S, S172D | 1410 | _ | _ | 0.193 | | 3.89 |
| CODA535 | L131T, S172D | 3850 | _ | _ | 2.65 | | 5.5 |
| CODA805 | Y115D, S170T | 30000 | | | 2.47 | | |
| CODA806 | Y115D, L131Q | 28000 | | | 0.145 | | |
| CODA807 | Y115D, L131E | 1790 | | | 0.095 | | |
| CODA952 | Y140I | 1740 | 0.311 | | | | |
| CODA1025 | R101F, L131G | 993 μM | 0.017 | | | | |
| CODA1027 | R101F, L131D | 3880 | 0.025 | | | | |
| CODA1039 | Y115E, Y210W | 4820 | 0.099 | | | | |
| CODA1045 | R101W, Y210V | 11800 | 0.0704 | | | | |
| CODA1047 | R101F, Y210V | 14400 | 0.159 | | | | |
| CODA1048 | R101F, Y210F | 4780 | 0.041 | | | | |
| CODA1053 | R101M, L131A | 1740 | 0.0036 | | | | |
| CODA1054 | R101M, L131F | 4740 | 0.0218 | | | | |
| COAD1055 | R101W,Y115E, Y210W | 3860 | 0.0106 | | | | |
| COAD1056 | R101F,Y115E, Y210W | 4180 | 0.042 | | | | |
| CODA1138 | W77F,R101F, L131D | 1900 | | 0.041 | | | |

| | | High-throughput Ephys EC50 (uM) | | | | | |
|---|---|---|---|---|---|---|---|
| | | HEK293 Cell Line | | | | | |
| **Name** | **Amino Acid Mutation(s) in SEQ ID NO: 33** | **Acetyl-choline** | **TC-5619** | **CNL002** | **Facini-cline** | ***TC-6987*** | ***AZD-0328*** |
| CODA1140 | R101F,L131N, S172D | 3640 | | 0.019 | | | |
| CODA1157 | Q139E,S172D | 7000 | | 0.011 | | | |
| CODA1173 | S172D,Y210W | 14000 | | 0.027 | | | |

**Table 22B: EC50 from high throughput electrophysiology**

| | | High-throughput Ephys EC50 (uM) | | | | |
|---|---|---|---|---|---|---|
| | | HEK293 Cell Line | | | | |
| **Name** | **Amino Acid Mutation(s) in SEQ ID NO: 33** | **Acetyl-choline** | **TC-5619** | **ABT-126** | **Varenicline** | ***CNL001*** |
| CODA967 | Y140R | 2230 | 0.0490 | | | |
| CODA1101 | Y115E, L131M | 20200 | | 0.1020 | | |
| CODA1167 | Q139W, S172D | 3310 | | 0.1650 | | |
| CODA1168 | Q139W, Y210W | 2180 | | 0.1120 | | |
| CODA1245 | Q139W, S172D, Y217I | 7377 | | | | 0.0134 |
| CODA1261 | Y115E, Q139E, S172D | 7613 | | | | 0.0910 |
| CODA1458 | R101F, L131G, Q139L, Y217F | 2555 | | | 0.0202 | |
| CODA1471 | Y115Q, S172D | 3725 | | | | 0.0210 |
| CODA1500 | L141M, S172D, Y210W | 1596 | | | | 0.0060 |
| CODA1507 | R101F, L131N, Y217F | 1008 | | | 0.5883 | |

**[0381]** These results show that all the mutant engineered receptors have reduced potency to acetylcholine as compared to wild type nAchRa7 or SEQ ID NO: 33 control. For instance, some of the engineered receptors have EC50 values that are several orders of magnitudes higher than the EC50 of wild type nAchRa7. Furthermore, the results show that some of the engineered receptors have increased potency to certain non-native ligands, as compared to the wild type receptor. For instance, the engineered receptor comprising the amino acid sequence of L131D, S172D in SEQ ID NO: 33 shows at least 10-fold increased potency towards AZD-0328 and RG-3487, as compared to the wild type control receptor. These results demonstrate that the engineered receptors may be used to decrease potency to acetylcholine,

while simultaneously retaining or increasing the potency to synthetic small molecule nAChα7 receptor agonists, which have been recognized as being safe and well tolerated in humans.

**[0382]** These results from electrophysiological methods provide confirmation of the EC50 values from the plate reader, and further confirm the decoupling of acetylcholine and non-native ligand responses of the engineered receptors disclosed herein.

**Example 4: Characterization of the Engineered Receptors using Manual Patch Clamp Electrophysiology**

**[0383]** Using whole cell manual patch clamp electrophysiology in HEK293 cells, rat DRG neurons and rat hippocampal neurons, the EC50 of the various engineered receptors disclosed herein to Ach and non-native ligands was measured using the Hill equation with peak current measurements from escalating doses of ligand. The rheobase shift was also measured in rat DRG neurons, which reflects the efficacy of the receptors. To calculate the rheobase, first, the amount of current that can produce an action potential was determined. Then the ligand was added, followed by a step wise increase in the current injected up to 700 pA.

**[0384]** For manual patch clamp experiments, Cells plated on coverslips were visualized at 10x and 40x on an inverted fluorescence microscope (Olympus). Recordings were made with an Axopatch 200B amplifier and Digidata 1550B (Molecular Devices) at RT using 3-6 MOhm glass patch electrodes (Sutter, BF150-86-10). Unless otherwise stated, recordings were made in extracellular solution (ECS) containing: 140 mM sodium chloride, 4 mM potassium chloride, 1 mM magnesium chloride, 2 mM calcium chloride, 10 mM HEPES, and 10 mM D-(+)-glucose (pH = 7.3 with sodium hydroxide, osmolarity = 305-315 mOsm). An 8-line reservoir (AutoMate Scientific) in combination with an 8-channel zero-dead-volume perfusion pencil (AutoMate Scientific) was used for perfusion. Data was acquired using pClamp software (Molecular Devices). Traces were either analyzed in Clampfit (Molecular Devices) or using custom Python code. Plotting and statistics were performed in GraphPad Prism.

**[0385]** For dose-response, ligand-induced currents were recorded in voltage clamp mode. For HEK cell experiments, recordings took place in the normal ECS with a cesium-based internal solution containing: 140 mM cesium chloride, 5 mM potassium fluoride, 2 mM Calcium chloride, 2 mM magnesium chloride, 10 mM HEPES, and 10 mM EGTA (pH = 7.2 with cesium hydroxide, osmolarity = 290-295 mOsm). For DRG neuron experiments, cells were patched in normal ECS and then switched to an NMDG-based ECS to prevent endogenous cation currents, containing: 145 mM N-methyl-D-glucamine, 4 mM potassium chloride, 0.5 mM magnesium chloride, 0.5 mM calcium chloride, 10 mM HEPES, and 10 mM

D-(+)-glucose (pH = 7.4 with hydrochloric acid, osmolarity = 305-315mOsm). Internal solution for DRG neurons contained: 145 mM cesium chloride, 1 mM magnesium chloride, 5 mM magnesium ATP, 10 mM HEPES, and 1 mM EGTA (pH = 7.2 with cesium hydroxide, osmolarity = 290-295 mOsm) Cells were held at -60mV and current across the membrane was recorded in gapfree mode at 3 kHz sampling rate, with Bessel filter at 1 kHz. Capacitance and series resistance were compensated. Drugs tested include acetycholine chloride and other synthetic compounds. Drug doses were applied for 1-10 sec until the peak of the current was observed, and washes occurred for at least 2 min between dose applications. The holding current was subtracted from the peak to find the current amplitude.

**[0386]** DRG rheobase experiments were performed in current clamp mode using a potassium-based internal solution containing: 100 mM potassium D-gluconate, 28 mM potassium chloride, 1 mM magnesium chloride hexahydrate, 5mM magnesium ATP, 10 mM HEPES , and 0.5 mM EGTA (pH = 7.2 with potassium hydroxide, osmolarity = 290-295 mOsm, calculated liquid junction potential = -13.5 mV). To measure DRG input resistance and rheobase, 500 ms current steps (-200 pA to 700 pA) were applied the membrane in the presence and absence of a compound. No holding current was applied to the membrane for cells included in the final datasets. Data were acquired at 10 kHz, with Bessel filter at 2 kHz and series resistance compensation at 100%. Rheobase was defined as the smallest depolarizing current injection required to cause an action potential to fire in the cell. In a subset of cells, the rheobase was ambiguous, since the action potential appeared to be graded in amplitude. In these cases, a polar plot of the trace clarified the rheobase. Input resistance was measured from the negative current injections, calculated as the difference in membrane potential at steady state divided by the current injection amplitude and then averaged across the four negative current steps for each recording. Resting membrane potential was measured from the first 100ms of each sweep, averaged for each recording, and corrected for liquid junction potential. One-way ANOVA with multiple comparisons was used to test for significance of difference in rheobase and input resistance between baseline, drug, and wash conditions.

**[0387]** Hippocampal rheobase experiments were performed in current clamp mode using a potassium-based internal solution containing: 115 mM potassium D-gluconate, 13 mM potassium chloride, 1 mM magnesium chloride hexahydrate, 5 mM magnesium ATP, 10 mM HEPES, and 0.5 EGTA mM (pH = 7.2 with potassium hydroxide, osmolarity = 290-295 mOsm, calculated liquid junction potential = -14.9 mV. NBQX (10 μM) and AP5 (50 μM) were included in the bath to block excitatory synaptic transmission. To measure HC input resistance and rheobase, 500 ms current steps (-200pA to 700pA) were applied the membrane in the

presence and absence of a compound. No holding current was applied to the membrane for cells included in the final datasets. Data acquisition settings and analysis methods were the same as for the DRG rheobase experiments.

**[0388]** Hippocampal spontaneous action potentials were recorded in current clamp mode using a potassium-based internal solution containing: 115 mM potassium D-gluconate, 13 mM potassium chloride, 1 mM magnesium chloride hexahydrate, 5 mM magnesium ATP, 10 mM HEPES, and 0.5 mM EGTA (pH = 7.2, osmolarity = 290-295 mOsm, calculated liquid junction potential = -14.9 mV). Spontaneous activity was recorded in gapfree mode, in the presence and absence of a compound. Data were acquired at 10 kHz, with Bessel filter at 2 kHz and series resistance compensation at 100%. No holding current was applied to the membrane. Data were analyzed using Clampfit as well as custom Python code (within-cell comparisons of the two methods did not reveal differences in results). For Clampfit, threshold-based event detection was run separately for each epoch to detect action potentials. For the custom Python code, a peak-finding package was used to automatically detect action potentials (SciPy). For both methods, minimum height, average height, maximum height were measured and frequency was calculated as the number of events in the epoch divided by the length of time of the epoch. For Clampfit, the resting membrane potential was measured at 3 points between events and averaged for each epoch. For resting membrane potential in Python, a median filter was applied to the trace and averaged for each epoch. For both methods, membrane potential was corrected for the calculated liquid junction potential. One-way ANOVA with multiple comparisons was used to test for significance between baseline, drug, and wash conditions.

**[0389]** The results are tabulated below in **Tables 23A - 27**. These results confirm that the engineered receptors disclosed herein have very low potency to Ach, but higher potency and efficacy to non-native ligands such as RG3487 and TC5619. *See* also, Example 6.

**Table 23A: Manual patch clamp based EC50 values using HEK293 cell line**

| | EC50 | | | |
|---|---|---|---|---|
| **Name** | ***TC-5619*** | ***CNL002*** | ***Facinicline/RG-3487*** | ***Acetylcholine*** |
| CODA71 | 0.03 μM | | 0.20 μM | 65 uM |
| CODA952 | | | | 1740 μM |
| CODA806 | | | 0.29 μM | >30,000 μM |
| CODA807 | | | 0.23 μM | 2200 μM |
| CODA1025 | 0.041 μM | | | 3853 μM |
| CODA1027 | 0.095 μM | | | 6412 μM |
| CODA1039 | 0.201 μM | | | 5400 μM |
| CODA1045 | 0.253 μM | | | >100,000 μM |

| | EC50 | | | |
|---|---|---|---|---|
| **Name** | ***TC-5619*** | ***CNL002*** | ***Facinicline/RG-3487*** | ***Acetylcholine*** |
| CODA1048 | 0.156 μM | | | 7341 μM |
| CODA1054 | 0.040 μM | | | 7426 μM |
| COAD1055 | 0.016 μM | | | 3899 μM |
| COAD1056 | 0.096 μM | | | 4460 μM |
| CODA1138 | | 0.033 μM | | 1963 μM |
| CODA1140 | | 0.018 μM | | 3134 μM |
| CODA1157 | | 0.032 μM | | 2521 μM |
| CODA1173 | | 0.039 μM | | 2782 μM |

**Table 23B: Manual patch clamp based EC50 values using HEK293 cell line**

| | EC50 | |
|---|---|---|
| **Name** | ***ABT-126*** | ***Varenicline*** |
| CODA1168 | 0.361 μM | |
| CODA1458 | | 0.0180 μM |

**Table 24: Manual patch clamp based EC50 values using Rat DRG Neurons**

| | EC50 | | |
|---|---|---|---|
| **Name** | **TC-5619** | **Facinicline/RG-3487** | **Acetylcholine** |
| CODA806 | | 0.29 μM | >30,000 μM |
| CODA1025 | 0.107 μM | | 9700 μM |
| CODA1027 | 0.377 μM | | >100,000 μM |
| CODA1039 | 0.649 μM | | >100,000 μM |
| CODA1045 | 0.268 μM | | >100,000 μM |
| CODA1048 | 0.203 μM | | 8552 μM |
| CODA1054 | 0.150 μM | | 11070 μM |
| COAD1055 | 0.056 μM | | 6825 μM |
| COAD1056 | 0.116 μM | | 7449 μM |

**Table 25: Manual patch clamp based EC50 values using Rat Hippocampal Neurons**

| | EC50 | |
|---|---|---|
| **Name** | **CNL002** | **Acetylcholine** |
| CODA1138 | 0.139 μM | 3369 μM |
| CODA1140 | 0.031 μM | |
| CODA1157 | 0.095 μM | 4669 μM |
| CODA1173 | 0.121 μM | 8461 μM |

**Table 26: Rheobase shift of rat DRG neurons using indicated ligand at EC90**

| | Rheobase Shift; Rat DRG Neurons | | |
|---|---|---|---|
| **Name** | **Baseline** | **+TC-5619 @ EC90** | **Facinicline/RG-3487 @ EC90** |
| CODA806 | 163 ± 28 pA | | 625 ± 35 pA |
| CODA1025 | 140 ± 19 pA | 560 ± 72 pA | |
| CODA1027 | 100 ± 23 pA | 638 ± 63 pA | |
| CODA1039 | 88 ± 9 pA | 453 ± 58 pA | |
| CODA1045 | 141 ± 22 pA | 700 ± 0 pA | |
| CODA1048 | 120 ± 19 pA | 590 ± 46 pA | |
| CODA1054 | 105 ± 13 pA | 673 ± 27 pA | |
| COAD1055 | 83 ± 7 pA | 562 ± 35 pA | |
| COAD1056 | 114 ± 12 pA | 632 ± 43 pA | |

**Table 27: Spontaneous Firing Rate and Evoked Rheobase Shift of Rat Hippocampal Neurons at EC90**

| **Firing Rate Spontaneous; Rat Hippocampal Neurons** | | |
|---|---|---|
| ***Sequence of the engineered receptor*** | ***Baseline*** | ***+CNL002 @ EC90*** |
| R101F_L131N_S172D in SEQ ID NO: 33 (CODA1140) | 3.29 ± 0.75 Hz | 0.71 ± 0.29 Hz |
| **Rheobase Shift Evoked; Rat Hippocampal Neurons** | | |
| ***Sequence of the engineered receptor*** | ***Baseline*** | ***+CNL002 @ EC90*** |
| R101F_L131N_S172D in SEQ ID NO: 33 (CODA1140) | 40.63 ± 10.50 pA | 296.9 ± 72.65 pA |

**Example 5: Characterization of the Localization of the Engineered Receptors**

**[0390]** The efficiency with which the engineered receptors disclosed herein are localized to the surface of the cells was evaluated using multiple methods. First, HA-tagged engineered receptors were expressed in HEK293T cells and their surface expression was monitored using fluorescently tagged HA antibodies. Second, fluorescently labelled α-bungarotoxin, which specifically binds to amino acids on the engineered receptors comprising LBD derived from nAchRa7, was used to bind to the engineered receptors. The use of both these methods, followed by flow cytometry, allowed corroboration of the surface localization of the engineered receptors.

**[0391]** Monoclonal antibodies anti-HA-PeCy7 (16B12) were purchased from Biolegend (San Diego, CA). The monoclonal antibody clone name is listed in parentheses. alpha-bungarotoxin conjugated to biotin, Alexa Fluor 647 were all purchased from Thermo/Fisher (Waltham MA). Briefly, HEK-293T were plated at 200,00 cells per well and

transfected with Fugene 6 the next day at a 1:3 ratio of DNA to Fugene. Cells were analyzed the day after transfection using flow cytometry. For flow cytometry analysis, transfected HEK293T cells were lifted using 0.05% Trypsin and 0.02% EDTA (Thermo/Fisher), washed and incubated in antibody (30min, 1:100) or α-bungarotoxin at (1 hour. 1:1000) in FACS Buffer (2% BSA, 1X PBS without Ca+ and Mg+, and 1X Penicillin Streptomycin). Cells were then washed in FACS buffer and then analyzed on a Sony SH800 FACS sorter (San Jose, CA). Subsequent analysis was done using FlowJo (San Jose, CA). Data presented are normalized to the % of cells positive for HA-tag fluorescence or α-bungarotoxin staining; and the median fluorescent intensity of the parental chimeric receptor comprising the amino acid sequence of SEQ ID NO: 33.

**[0392]** **FIG. 5** and **Table 28** show % of HA-tag positive cells that are expressing the engineered receptors, normalized to control cells expressing the amino acid sequence of SEQ ID NO: 33 ("Normalized HA %"); and the % of α-bungarotoxin positive cells that are expressing the engineered receptors, normalized to control cells expressing the amino acid sequence of SEQ ID NO: 33 ("Normalized AB %"). They also show the median fluorescent intensity (MFI) of a cell expressing the engineered receptors, normalized to control cells expressing the amino acid sequence of SEQ ID NO: 33, as evaluated using anti-HA antibodies ("Normalized HA MFI") or fluorescently labeled α-bungarotoxin conjugated to Alexa Fluor 647 ("Normalized AB MFI"). The different point mutations can influence the detection of both HA and alpha-bungarotoxin by flow cytometry.

**Table 28: Surface Localization of the Engineered Receptors**

| Name | Normalized HA % | Normalized HA MFI | N | Normalized AB % | Normalized AB MFI | N |
|---|---|---|---|---|---|---|
| CODA282 | 0.91 | 0.74 | 2 | 1.23 | 1.04 | 2 |
| CODA283 | 1.16 | 1.00 | 2 | 1.24 | 1.31 | 2 |
| CODA333 | 1.42 | 0.76 | 2 | 1.28 | 1.20 | 2 |
| CODA335 | 2.00 | 0.88 | 2 | 1.65 | 1.32 | 3 |
| CODA339 | 1.59 | 0.79 | 2 | 0.84 | 1.41 | 3 |
| CODA342 | 1.83 | 0.74 | 2 | 0.61 | 1.24 | 3 |
| CODA377 | 0.36 | 0.61 | 2 | 0.50 | 0.90 | 4 |
| CODA409 | 0.96 | 1.09 | 2 | 0.88 | 0.62 | 4 |
| CODA534 | 0.47 | 1.10 | 8 | 0.44 | 0.91 | 3 |
| CODA535 | 0.33 | 0.86 | 9 | 0.43 | 0.72 | 2 |
| CODA536 | 0.40 | 0.87 | 9 | 0.48 | 0.87 | 2 |

| Name | Normalized HA % | Normalized HA MFI | N | Normalized AB % | Normalized AB MFI | N |
|---|---|---|---|---|---|---|
| CODA805 | 0.70 | 0.94 | 4 | 0.56 | 0.93 | 4 |
| CODA806 | 0.36 | 0.83 | 4 | 0.33 | 2.04 | 5 |
| CODA807 | 0.91 | 1.00 | 4 | 0.78 | 1.14 | 4 |
| CODA952 | 1.41 | 1.53 | 2 | 0.54 | 2.64 | 2 |
| CODA1025 | 0.98 | 0.63 | 2 | 0.46 | 0.90 | 2 |
| CODA1027 | 0.61 | 0.49 | 2 | 0.50 | 0.68 | 2 |
| CODA1058 | 1.44 | 0.68 | 2 | 0.34 | 1.08 | 2 |
| CODA1045 | 1.09 | 0.87 | 11 | 0.03 | 0.35 | 12 |
| CODA1047 | 1.13 | 0.65 | 4 | 0.03 | 0.38 | 4 |
| CODA1048 | 1.05 | 0.61 | 4 | 0.55 | 0.57 | 4 |
| CODA1053 | 1.31 | 0.97 | 10 | 0.91 | 0.90 | 10 |
| CODA1054 | 0.96 | 0.88 | 11 | 0.82 | 0.82 | 12 |
| CODA1072 | 1.07 | 0.78 | 9 | 0.51 | 0.69 | 9 |
| CODA1073 | 1.22 | 0.56 | 4 | 0.44 | 0.70 | 4 |

*Abbreviations: MFI- Mean Fluorescence Intensity; AB- α-bungarotoxin

**[0393]** The results show that the mutations in the engineered receptors disclosed herein affect the localization to the surface of the cell. While the localization of some double mutants is comparable to that of the parental chimera (SEQ ID NO: 33), others have diminished cell surface localization compared to the parental chimera (SEQ ID NO: 33). For instance, the engineered receptor CODA409 shows comparable localization to the parental chimera as evaluated by the HA tag. Moreover, the majority of the engineered receptors show comparable localization to the parental chimera by both techniques, that is, as evaluated by the HA tag as well as the α-bungarotoxin. On the other hand, certain mutations may affect the binding to α-bungarotoxin.

*Localization of the Engineered Receptors in Neuron Cells*

**[0394]** To determine the efficiency with which the engineered receptors disclosed herein are localized to the surface of the neuron cells, immunocytochemistry was used in rat DRG neurons and rat hippocampal neurons.

**[0395]** *Primary neuron culture:* For dorsal root ganglion (DRG) neuron experiments, adult rat lumbar ganglia were harvested and dissociated according to standard protocols, cultured on glass coverslips coated with poly-L-lysine and mouse laminin, transduced with

human synapsin-driven, ires-GFP-containing lentiviral vectors, and processed for immunocytochemistry. For hippocampal neuron experiments, hippocampi were harvested from embryonic day 18 rat pups and dissociated according to standard protocols, cultured on glass coverslips coated with poly-L-lysine, transduced with human synapsin-driven, ires-GFP-containing lentiviral or AAV6 vectors, and processed for immunocytochemistry. 5-fluoro-deoxyuridine was added to hippocampal cultures to inhibit glial growth. The ires-GFP-containing lentiviral vectors either encoded for HA tagged engineered receptors with the indicated sequence, or were empty control ires-GFP vectors.

**[0396]** *Immunocytochemistry and quantification:* Coverslips were rinsed in protein-free HEPES-based extracellular buffer, and non-viable cells stained with Live-or-Dye 350/448 (Biotium) at room temperature. After rinsing, cells were stained for surface HA tag with rabbit anti-HA antibody at 4C. Cells were fixed at room temperature in 4% paraformaldehyde, blocked and permeabilized with 5% normal goat serum / 0.1% triton X-100, and stained overnight at 4C for total HA tag (mouse anti-HA antibody) and GFP. After washing, cells were incubated with appropriate fluorescent secondary goat or donkey antibodies, washed, mounted and imaged via 4-color fluorescence microscopy. Images were analyzed with custom scripts (ImageJ) to identify viable neuronal soma and axon / dendritic compartments, and to quantify surface and total HA immunoreactivity present in these structures. All data are expressed as mean +/- SEM of individual coverslips, from multiple independent cultures as indicated. For results of DRG neurons transduced with lentiviral vectors, the values for cells transduced with empty vector are listed. For all other results, the values for cells transduced with empty vector are subtracted from all measurements as part of experimental background correction.

**[0397]** The results are provided in **FIG. 6A** and **FIG. 6B**. The fluorescence signal from the HA tagged engineered receptor in the soma of the neuron, or in the processes (including pseudo-axons, and dendrites) is presented as a fraction of the corresponding fluorescence signal from that of the wild type GLRA1 receptor. The "total" value indicates the fluorescence signal of the engineered receptor as a fraction of the corresponding fluorescence signal from that of the wild type GLRA1 obtained from the whole cell after permeabilization. The "surface" value indicates the fluorescence signal of the engineered receptor as a fraction of the corresponding fluorescence signal from that of the wild type GLRA1 obtained from just the cell surface in cells without permeabilization. Blank cells indicate data not yet available; (0) indicates that the value is below assay detection threshold.

**[0398]** These results indicate that the engineered receptors are capable of being expressed in neurons and localize to the cell surface. And some of the engineered receptors are more effectively expressed and localized to the soma.

**Example 6: Characterization of the CR-11 engineered receptor**

**[0399]** The high throughput electrophysiology platform indicated that the potency of the engineered receptor comprising an amino acid sequence with amino acid substitutions of Y115D and L131Q in SEQ ID NO: 33, named CR-11, to acetylcholine is over 500 fold reduced as compared to the wild type receptor, while its potency to RG-3487 is increased by over 10-fold.

**[0400]** Using whole cell manual patch clamp electrophysiology, in both HEK293 cells and cultured rat dorsal root ganglion (DRG) sensory neurons, it was confirmed the engineered receptor comprising an amino acid sequence with amino acid substitutions of Y115D and L131Q in SEQ ID NO: 33 is essentially insensitive to acetylcholine, but way more sensitive to RG-3487, as compared to the wildtype nAchRα7 receptor. See **FIG. 2A** – **FIG. 2B**.

**[0401]** In HEK293 cells, the $EC_{50}$ of the engineered receptor comprising an amino acid sequence with amino acid substitutions of Y115D and L131Q in SEQ ID NO: 33 to ACh could not be determined because almost no current could be generated, even at concentration of Ach up to 100 mM. In contrast, the $EC_{50}$ of the wildtype nAchRα7 receptor to ACh is 42.4 μM (**FIG. 2A**). Further confirming the high throughput data, the manual patch clamp electrophysiology results also shows that the engineered receptor comprising an amino acid sequence with amino acid substitutions of Y115D and L131Q in SEQ ID NO: 33 is more sensitive to RG-3487 (SA-2) (with an $EC_{50}$ = 0.3 μM), as compared to the wildtype nAchα7 receptor ($EC_{50}$ =2.9 μM) (**FIG. 2B**).

**[0402]** In cultured adult rat DRG neurons expressing the engineered receptor comprising an amino acid sequence with amino acid substitutions of Y115D and L131Q in SEQ ID NO: 33, application of RG-3487 (SA-2) generated chloride current in a dose dependent manner (**FIG. 3**), although such a current is not observed in non-transduced cells.

**[0403]** Additionally, aactivation of the engineered receptor comprising an amino acid sequence with amino acid substitutions of Y115D and L131Q in SEQ ID NO: 33 with RG-3487 (SA-2) at 3 μM concentration in transduced rat DRG neurons reversibly inhibits current injection evoked action potentials (**FIG. 4A**) with an approximate 3x increase in the current required to elicit action potentials (n=7). Acetylcholine at 3.0 mM had no effect on evoked

action potentials in transduced neurons (n=4) (**FIG. 4B**). These results show that expression of the engineered receptor comprising an amino acid sequence with amino acid substitutions of Y115D and L131Q in SEQ ID NO: 33 in DRG neurons can inhibit evoked action potential.

**Example 7. Peak current measurements of engineered receptors using IonFlux and manual patch clamp**

**[0404]** ***Peak current and dose response measurements using IonFlux*:**

**[0405]** HEK 293 cells were routinely subcultured in DMEM with 10% fetal bovine serum (ThermoFisher Scientific). Cells were plated in 15 cm dishes 24 hours prior to transfection with Fugene 6 (Promega, E2691) and plasmid DNA (total of 20 μg DNA). 24 hours post transfection, cells were lifted with Accutase (Sigma, A6964) then resuspended in serum free media (CHO-S-SFM II, ThermoFisher Scientific 12052 with 25 mM HEPES, ThermoFisher Scientific, 15630080) to rest for 30-90 min before being loaded onto IonFlux (Fluxion Biosciences) for experiments. Recordings were made in extracellular solution (ECS) containing (in mM): 140 sodium chloride (S7653), 4 potassium chloride (P9333), 1 magnesium chloride (M2670), 2 calcium chloride (C3881), 10 HEPES (54457), and 10 D-(+)-glucose (G7528) (pH = 7.3 with sodium hydroxide, osmolarity = 305-315 mOsm). Intracellular solution (ICS) contains (in mM): 140 cesium chloride (289329), 5 potassium fluoride (60238), 2 Calcium chloride (P9333), 2 magnesium chloride (M2670), 10 HEPES (54457), and 10 EGTA (3777) (pH = 7.2 with cesium hydroxide, osmolarity = 290-295 mOsm).

**[0406]** Drug-induced currents were recorded in ensemble mode (20 cells are patched per recording channel). Cells were held at -60 mV and current across the membrane was recorded at 1 kHz sampling rate. Drugs tested include acetycholine chloride (A6625) and other synthetic compounds. Drug doses were applied for 5 sec with a 2 min wash between dose applications unless otherwise stated. The holding current was subtracted from the peak to find the current amplitude. Doses were applied in half-log increments, increasing in concentration until the peak current amplitude plateaued. For each ensembled channel, amplitudes were normalized to the largest current. Normalized current amplitudes were averaged across channels and data were fit to a dose-response curve using a nonlinear regression model (Y= Bottom + (Top-Bottom)/(1+EC50/X)^Hillslope). The maximum peak currents (absolute amplitude) from all the ensembled channels were averaged for each receptor and normalized to the mean maximum peak current from CODA71HA that was transfected and experimented in the same batch.

**[0407]** ***Peak current and dose response measurements using manual patch clamp*:**

**[0408]** **Cell culture:** HEK 293 cells were routinely subcultured in DMEM with 10% fetal bovine serum (ThermoFisher Scientific). Cells were plated in six-well dishes 24 hours prior to transfection with Fugene 6 (Promega, E2691) and plasmid DNA (total of 1 μg DNA). 24 hours post transfection, cells were replated onto 10 mm PDL and collagen coated coverslips, before being used for patch clamp experiments 24–48 hours later. For dorsal root ganglion neuron experiments, adult rat lumbar ganglia were harvested and dissociated according to standard protocols, cultured on glass coverslips coated with poly-L-lysine and mouse laminin, transduced on DIV1 with ires-GFP-containing lentiviral or AAV6 vectors, and processed for immunocytochemistry on DIV7. For hippocampal neuron experiments, hippocampi were harvested from embryonic day 18 rat pups or postnatal day 0 mouse pups and dissociated according to standard protocols. Cells were cultured on glass coverslips coated with poly-L-lysine, transduced on DIV3 with ires-GFP-containing lentiviral or AAV6 vectors, and processed for immunocytochemistry on DIV14 (surface expression experiments) or stained with calcein violet and imaged on DIV16 (neuronal viability assays). 5-fluoro-deoxyuridine was added to hippocampal cultures on DIV3 (rat E18) or DIV7 (mouse P0) to inhibit glial growth. For surface expression experiments employing lentiviral expression, cultures were transduced at MOI = 20 (DRG) or MOI = 5 (hippocampal) with human synapsin promoter-driven lentiviral vectors. For lentiviral viability assays, cultures were transduced at MOI = 2 (DRG) or MOI = 1 (hippocampal) with ubiquitin promoter-driven lentiviral vectors. For all experiments employing AAV6 expression, cultures were transduced at MOI = 2e5 (rat DRG and E18 hippocampal) or MOI = 3e8 (mouse p0 hippocampal) with human synapsin promoter-driven AAV6 vectors.

**[0409]** **Whole-cell patch clamp recording protocol:** Cells plated on coverslips were visualized at 10x and 40x on an inverted fluorescence microscope (Olympus). Recordings were made with an Axopatch 200B amplifier and Digidata 1550B (Molecular Devices) at RT using 3-6 MOhm glass patch electrodes (Sutter, BF150-86-10). Chemicals were acquired from Sigma except for TC-5619, which was manufactured by Attenua/Targacept. Unless otherwise stated, recordings were made in extracellular solution (ECS) containing (in mM): 140 sodium chloride (S7653), 4 potassium chloride (P9333), 1 magnesium chloride (M2670), 2 calcium chloride (C3881), 10 HEPES (54457), and 10 D-(+)-glucose (G7528) (pH = 7.3 with sodium hydroxide, osmolarity = 305-315 mOsm). An 8-line reservoir (AutoMate Scientific) in combination with an 8-channel zero-dead-volume perfusion pencil (AutoMate Scientific) was used for perfusion. Data was acquired using pClamp software (Molecular Devices). Traces were either analyzed

in Clampfit (Molecular Devices) or using custom Python code. Plotting and statistics were performed in GraphPad Prism.

**[0410]** **Dose-response experiments:** Drug-induced currents were recorded in voltage clamp mode. For HEK cell experiments, recordings took place in the normal ECS with a cesium-based internal solution containing (in mM): 140 cesium chloride (289329), 5 potassium fluoride (60238), 2 Calcium chloride (P9333), 2 magnesium chloride (M2670), 10 HEPES (54457), and 10 EGTA (3777) (pH = 7.2 with cesium hydroxide, osmolarity = 290-295 mOsm). For DRG neuron experiments, cells were patched in normal ECS and then switched to an NMDG-based ECS to prevent endogenous cation currents, containing (in mM): 145 N-methyl-D-glucamine (M2004), 4 potassium chloride (P9333), 0.5 magnesium chloride (M2670), 0.5 calcium chloride (C3881), 10 HEPES (54457), and 10 D-(+)-glucose (G7528) (pH = 7.4 with hydrochloric acid, osmolarity = 305-315mOsm). Internal solution for DRG neurons contained (in mM): 145 cesium chloride (289329), 1 magnesium chloride (M2670), 5 magnesium ATP (A9187), 10 HEPES (54457), and 1 EGTA (3777) (pH = 7.2 with cesium hydroxide, osmolarity = 290-295 mOsm). Cells were held at -60mV and current across the membrane was recorded in gapfree mode at 3 kHz sampling rate, with Bessel filter at 1 kHz. Capacitance and series resistance were compensated. Drugs tested include acetycholine chloride (A6625) and other synthetic compounds. Drug doses were applied for 1-10 sec until the peak of the current was observed, and washes occurred for at least 2min between dose applications. The holding current was subtracted from the peak to find the current amplitude. Doses were applied in half-log increments, increasing in concentration until the peak current amplitude plateaued. For each cell, amplitudes were normalized to the largest current. Normalized current amplitudes were averaged across cells and data were fit to a dose-response curve using a nonlinear regression model (Y= Bottom + (Top-Bottom)/(1+EC50/X)^Hillslope) . The maximum peak currents (absolute amplitude) from all the cells for each receptor were averaged and within-batch comparisons were performed to control for batch effects.

**[0411]** **Rheobase experiments:**

**[0412]** *DRG neurons*: DRG rheobase experiments were performed in current clamp mode using a potassium-based internal solution containing (in mM): 100 potassium D-gluconate (G4500), 28 potassium chloride (P9333), 1 magnesium chloride hexahydrate (M2670), 5 magnesium ATP (A9187), 10 HEPES (54457), and 0.5 EGTA (3777) (pH = 7.2 with potassium hydroxide, osmolarity = 290-295 mOsm, calculated liquid junction potential = -13.5 mV). To measure DRG input resistance and rheobase, 500 ms current steps (-200 pA to 700 pA) were applied the membrane (-400 pA to 700 pA in an early version of the protocol) in

the presence and absence of a compound. No holding current was applied to the membrane for cells included in the final datasets. Data were acquired at 10 kHz, with Bessel filter at 2 kHz and series resistance compensation at 100%. Rheobase was defined as the smallest depolarizing current injection required to cause an action potential to fire in the cell. In a subset of cells, the rheobase was ambiguous, since the action potential appeared to be graded in amplitude. In these cases, a polar plot of the trace clarified the rheobase. Input resistance was measured from the negative current injections, calculated as the difference in membrane potential at steady state divided by the current injection amplitude and then averaged across the four negative current steps for each recording. Resting membrane potential was measured from the first 100ms of each sweep, averaged for each recording, and corrected for liquid junction potential. One-way ANOVA with multiple comparisons was used to test for significance of difference in rheobase and input resistance between baseline, drug, and wash conditions.

**[0413]** *Hippocampal neurons*: Hippocampal rheobase experiments were performed in current clamp mode using a potassium-based internal solution containing (in mM): 115 potassium D-gluconate (G4500), 13 potassium chloride (P9333), 1 magnesium chloride hexahydrate (M2670), 5 magnesium ATP (A9187), 10 HEPES (54457), and 0.5 EGTA (3777) (pH = 7.2 with potassium hydroxide, osmolarity = 290-295 mOsm, calculated liquid junction potential = -14.9 mV. NBQX (10 μM) and AP5 (50 μM) were included in the bath to block excitatory synaptic transmission. To measure HC input resistance and rheobase, 500 ms current steps (-200pA to 700pA) were applied the membrane in the presence and absence of a compound. No holding current was applied to the membrane for cells included in the final datasets. Data acquisition settings and analysis methods were the same as for the DRG rheobase experiments.

**[0414]** **Hippocampal spontaneous action potential experiments:** Hippocampal spontaneous action potentials were recorded in current clamp mode using a potassium-based internal solution containing (in mM): 115 potassium D-gluconate (G4500), 13 potassium chloride (P9333), 1 magnesium chloride hexahydrate (M2670), 5 magnesium ATP (A9187), 10 HEPES (54457), and 0.5 EGTA (3777) (pH = 7.2, osmolarity = 290-295 mOsm, calculated liquid junction potential = -14.9 mV). Spontaneous activity was recorded in gapfree mode, in the presence and absence of a compound. Data were acquired at 10 kHz, with Bessel filter at 2 kHz and series resistance compensation at 100%. No holding current was applied to the membrane. Data were analyzed using Clampfit as well as custom Python code (within-cell comparisons of the two methods did not reveal differences in results). For Clampfit, threshold-based event detection was run separately for each epoch to detect action potentials. For the

custom Python code, a peak-finding package was used to automatically detect action potentials (SciPy). For both methods, minimum height, average height, maximum height were measured and frequency was calculated as the number of events in the epoch divided by the length of time of the epoch. For Clampfit, the resting membrane potential was measured at 3 points between events and averaged for each epoch. For resting membrane potential in Python, a median filter was applied to the trace and averaged for each epoch. For both methods, membrane potential was corrected for the calculated liquid junction potential. One-way ANOVA with multiple comparisons was used to test for significance between baseline, drug, and wash conditions.

**Example 8. Micro electrode array measurements of the engineered receptors**

**[0415]** Primary hippocampal cultures were obtained from C57/Bl6 mice at day E17.5. Mice were sacrificed by inhalation of $CO_2$ and embryos removed immediately. Embryonic brains were dissected in ice cold HBSS supplemented with 0.6% glucose and 5 mM HEPES at pH 7.4. The hippocampus of each brain was extracted and rinse with HBSS twice then treated with 0.25% trypsin for 15 minutes at 37°C. Cells were then washed three time in HBSS, resuspended in Neurobasal media supplemented with 10% FBS and 1% PenStrep and mechanically dissociated by gentle pipetting with glass Pasteur pipette. Cells we plated at a density of 60,000 cell per well in a 24 well microelectrode array plate (Axion Biosystems) for 4 hours. The plating media was then replaced with Neurobasal media, 1x B-27, 1% PenStrep, 1 mM Glutamax, 10 mM HEPES pH 7.4

**[0416]** Cells were maintained in a humidified 5% CO2 incubator and 30% of the media was replaced every 2-3 days. On day in vitro (DIV) 1, cells were transduced with either AAV6 or AAV9 carrying the CODA receptor of interested under control of the hSYN promoter. On DIV 21 the mean firing rate (MFR) of each well was determined for 10 minutes prior to drug administration and then again 50-60 minutes post dose in a Maestro Edge microectrode array system. Axion Biosystems). Percent inhibition of the MFR in each well was calculated as 100-[(post dose MFR/pre-dose MFR)*100].

**Example 9. Characterization of engineered receptors in IPSC-derived neurons**

**[0417]** Differentiation protocols to generate IPSC-derived Aβ neurons are developed. The following markers are used to define the cells as Aβ neurons; expression of Neurofilament 200 (NF200), which delineates myelinated primary afferent neurons (Basbaum et al, 2009), Piezo 2, a marker of Low Threshold Mechanoreceptor sensory neurons (LTMRs) (Ranade et

al., 2014) and TLR5, a toll-like receptor that reportedly also marks Aβ fibers (Xu et al., 2015). The characterization will also include evaluation for absence of the nociceptor specific marker TrpV1, which is expressed in many C and Aβ fibers (Caterina et al., 1997), prostatic acid phosphatase, which delineates non-peptidergic unmyelinated afferents (Zylka et al., 2009), and NaV1.1, a marker of Aβ nociceptive neurons. IPSC-derived neurons that meet the above criteria will be further characterized as either rapidly adapting LTMRs, based on expression of c-Ret and MafA/C-Maf, or slowly adapting LTMRs, based on expression of TrkB and Shox2, in the absence of c-Ret expression (Koch et al., 2018).

**[0418]** (1) Confirm that cells meeting the above expression marker criteria of Aβ neurons have electrophysiology properties characteristic of non-nociceptive sensory neurons. These properties include generation of current in response to ligand and that direct current injection evokes action potentials.

**[0419]** (2) Confirm presence of chloride currents in neurons transduced with select chemogenetic receptors (engineered receptors). Cells are transduced with a lentivirus vector encoding an HA-tagged chemogenetic receptor(s) with an IRES GFP to identify transduced cells via GFP fluorescence. Chloride currents in response to Synthetic Agonists are detected in voltage clamp mode with NMGD+ as the internal/external fluid cation. As NMDG+ is impermeable to cation channels, its inclusion eliminates any endogenous nAchRα7 cation currents in response to the Synthetic Agonists studied. The EC50 for Chemogenetic Receptor-Synthetic Agonist pairs is then determined. Following recordings, expression and cell surface localization of the receptor(s) are evaluated by fluorescence microscopy in permeabilized and non-permeabilized cells using antibodies directed at the HA tag.

**[0420]** (3) Assess ability to inhibit current injection evoked action potentials. Cells are transduced with a lentivirus vector encoding an HA-tagged chemogenetic receptor(s) with an IRES GFP to identify transduced cells via GFP fluorescence. In current clamp mode, by escalating application of current until the cell membranes depolarizes, the rheobase in the presence and absence of the synthetic agonist is determined. Results are compared to cells that are transduced with GFP only.

**[0421]** (4) Assess impact on input resistance. Cells are transduced with a lentivirus vector encoding an HA-tagged chemogenetic receptor(s) with an IRES GFP to identify cells via GFP fluorescence. In current clamp mode, to calculate the input resistance, sub threshold current is injected and resulting change in membrane voltage is determined. Results are compared to cells that are transduced with GFP only.

**[0422]** (5) Assess impact on resting membrane potential in the presence and absence of synthetic agonist. Cells are transduced with a lentivirus vectors encoding an HA-tagged chemogenetic receptor(s) with an IRES GFP to identify transduced cells via GFP fluorescence. In voltage clamp mode the resting membrane potential is determined in the presence and absence of the synthetic agonist. Results are compared to cells that are transduced with GFP only.

**[0423]** The above electrophysiology properties in the IPSC-derived Aβ neurons are compared with those in IPSC-derived C-fiber neurons as well as adult rat DRG neurons. In addition, as biochemical changes in the injured Aβ fiber afferents can contribute to spontaneous pain in neuropathic states, the electrophysiological properties of the IPSC derived Aβ neurons is investigated following injury in vitro. To produce the injuries *in vitro,* cells are harvested and re-plated after processes have been extended in culture. The re-plating process severs the processes, mimicking an axonal injury. At various time point post injury the cells are evaluated for various electrophysiology properties including: generation of spontaneous action potentials, changes in resting membrane potentials and changes in the rheobase. The effect of the chemogenetic receptors are evaluated in the injured state as well.

**Example 10. Assessing the efficacy of engineered receptors to treat disease in animal models**

**[0424]** The engineered receptors disclosed herein are assessed for their ability to provide analgesia in a rat model of neuropathic pain following administration of a small molecule ligand. AAV expression cassettes containing a human synapsin-1 (hSYN) promoter linked to polynucleotides encoding either a wild type α7-nAChR, or any one of the engineered chimeric receptors disclosed herein are constructed using standard molecular biology techniques.

**[0425]** These AAV expression cassettes are subcloned into AAV bacmids, purified, transfected into Sf9 insect cells to produce recombinant baculovirus, and then amplified. Sf9 cells are double infected with the amplified recombinant baculovirus containing with either the wild type α7-nAChR, or any one of the engineered chimeric receptors cassettes described above, and another recombinant baculovirus containing the Rep and AAV6 (Y705+731F+T492V) Cap genes to produce recombinant AAV vectors. The viral vectors are purified, viral titer determined using qPCR, and SDS-PAGE used to verify the purity of AAV vectors.

**[0426]** *Behavioral experiments and pain models:* To produce mechanical hypersensitivity in a model that mimics a neuropathic pain condition, the spared nerve injury (SNI) model (a validated model of mechanical allodynia) is used (Shields et al., 2003, The Journal of Pain, 4, 465-470). This model is produced by the sectioning of the common peroneal and the sural nerves and isolating the tibial branch. Mechanical withdrawal threshold is assessed by placing rats on an elevated wire-mesh grid and stimulating the plantar surface of the hind paw with von Frey filaments.

**[0427]** *AAV injection into the spinal cord of rats:* A dorsal hemilaminectomy is made at the level of the lumbar enlargement to expose two segments (about 1.5–2 mm) of lumbar spinal cord, after which the dura mater is incised and reflected. The viral solution is loaded into a glass micropipette (prefilled with mineral oil). The micropipette is connected to a manual micro-injector mounted on a stereotactic apparatus. The viral solution is targeted to the dorsal horn (left side). Along the rostro-caudal axis within the exposed region, 6 injections of 240 nl each are performed, in an equidistant linear fashion. After each injection, 1 min of resting time is observed and then the muscle layer is sutured, the skin closed with staples, and the animals were allowed to recover with heated-pad before they were returned to their home cages. Animals are perfused for histological analysis after the last behavior test.

**[0428]** *AAV intraganglionic injections into the dorsal root ganglion (DRG) of rats:* The injection is performed with a borosilicate glass capillary (0.78/1 mm internal/external diameters) pulled to a fine point, attached by polyethylene tubing (0.4/0.8 mm internal/ external diameters) to a syringe mounted in a microinjection pump. The needle is mounted on an extended arm of a stereotaxic frame swung to the outside (used to hold and manipulate the needle only). Tubing, syringe, and needle are all filled with water. One microliter air is taken up into the needle followed by 3 μL of the viral vector solution. The needle is loaded separately with this volume for each injection. Animals are anesthetized prior to surgery. Following an incision along the dorsal midline, the L4 and L5 DRG are exposed by removal of the lateral processes of the vertebrae. The epineurium lying over the DRG is opened, and the glass needle inserted into the ganglion, to a depth of 400 μm from the surface of the exposed ganglion. After a 3-minute delay to allow sealing of the tissue around the glass capillary tip, 1.1 μL virus solution was injected at a rate of 0.2 μL/minute. After a further delay of 2 minutes, the needle is removed. The L4 ganglion is injected first followed by the L5 ganglion. The muscles overlying the spinal cord are loosely sutured together with a 5-0 suture and the wound closed. Animals are allowed to recover at 37 °C and received postoperative analgesia.

**[0429]** *AAV intrathecal injections in rats:* Rats are first anesthetized and then placed vertically with their head fixed in a stereotaxic frame. An incision is made in the base of the neck to expose the groove in the nuchal crest. An incision is made (1-2 mm) in the cisternal membrane to a depth such that cerebrospinal fluid leaks out. A 4 cm 32 G intrathecal catheter is then slowly inserted in the direction of the lumbar spinal cord and skin is closed by suture around the catheter. The rats are then allowed to recover. Rats are then anesthetized and the vector (6 μL) is administered. The catheter is flushed with 6 μL of PBS and then removed and rats allowed to recover.

**[0430]** *Effects of administration:* This SNI model is produced by the sectioning of the common peroneal and the sural nerves and isolating the tibial branch of the rat. The up-down method of Chaplan & Yaksh is used to determine mechanical thresholds before the injection of the AAV.hSYN-α7-nAChR/GlyRα1 into the spinal cord, DRG, or intrathecal space. Three weeks after unilateral vector injection, animals are tested again to verify that their mechanical withdrawal thresholds do not change. Motor coordination is also tested before and after injection, using an accelerating rotarod (Stoelting, USA) at a maximum speed of 33 rpm. The duration that the rat spends on the rotarod is recorded, with a cut-off at 300 sec. Each rat goes through three training trials and is tested two hours later.

**[0431]** Subsequently, half of the rats in each chimera cohort are administered a single IP injection of AZD-0328 or Facinicline and mechanical thresholds tested using the up-down method at 1, 2, 5, 7, and 13 days post IP injection. On the third day, when the thresholds has returned to post-injury baseline, AZD-0328 is again injected IP and again a recovery to non-injury baseline thresholds is observed. These animals are followed for 48 hours. Animals are then perfused for histology.

**Example 11: Treatment of a patient suffering from chronic pain**

**[0432]** In a non-limiting example, a patient suffering from chronic radicular pain is treated using the compositions and methods disclosed herein. The patient is treated on Day One with $10^{13}$ vector genomes of AAV.hSYN operably linked to a polynucleotide encoding any one of the engineered receptors disclosed herein in a volume of 1.0 mL delivered directly into one or more dorsal root ganglia (i.e., intraganglionic convection-enhanced delivery into lumbar, cervical, or thoracic DRGs). In this example, the AAV vector encodes any one of the engineered receptors disclosed herein under the control of the human Synapsin-1 (SYN1) promoter for selective neuronal expression. Two weeks post-injection, the patient returns to the clinic for a prescription for AZD-0328 or another non-native ligand. The patient self-

administers 0.1 mg/kg AZD-0328 or another non-native ligand orally as needed (i.e., during a pain episode).

**Example 12. Treatment of a patient suffering from chronic pain**

**[0433]** In a non-limiting example, a patient suffering from chronic craniofacial pain (*e.g.* trigeminal neuralgia or termporomandibular joint dysfunction) is treated using the compositions and methods disclosed herein. The patient is treated on Day One with $10^{13}$ vector genomes of AAV.hSYN operably linked to a polynucleotide encoding any one of the engineered receptors disclosed herein in a volume of 0.150 mL delivered directly into the trigeminal ganglion (i.e., intraganglionic convection enhanced delivery). In this example, the AAV vector encodes any one of the engineered receptors disclosed herein under the control of the human Synapsin-1 (SYN1) promoter for selective neuronal expression. Two weeks post-injection, the patient returns to the clinic for a prescription for AZD-0328 or another non-native ligand. The patient self-administers 0.1 mg/kg AZD-0328 or another non-native ligand orally as needed (i.e., during a pain episode).

**Example 13. Treatment of a patient suffering from obesity**

**[0434]** In a non-limiting example, a patient suffering from obesity is treated using the compositions and methods disclosed herein. The patient is treated on Day One with $10^{13}$ vector genomes of AAV. Ghrelin operably linked to a polynucleotide encoding any one of the engineered receptors disclosed herein in a volume of 1.0 mL delivered directly into the gastric branch of the vagus nerve (i.e., intraneural). In this example, the AAV vector encodes the engineered receptor under the control of the human Ghrelin promoter for selective neuronal expression. Two weeks post-injection, the patient returns to the clinic for a prescription for AZD-0328 or another non-native ligand. The patient self-administers 0.1 mg/kg AZD-0328 or another non-native ligand orally daily for excess weight loss (i.e. for apetitite suppression).

**Example 14. Treatment of a patient suffering from obesity**

**[0435]** In a non-limiting example, a patient suffering from obesity is treated using the compositions and methods disclosed herein. The patient is treated on Day One with $10^{13}$ vector genomes of AAV-TRPV1 operably linked to a polynucleotide encoding any one of the engineered receptors disclosed herein in a volume of 1.0 mL delivered directly into the dorsal root ganglia innervating the pancreas (i.e., intragangionic). In this example, the AAV vector encodes the engineered receptor under the control of the human TRPV1 promoter for selective neuronal expression in nociceptors. Two weeks post-injection, the patient returns to the clinic

for a prescription for AZD-0328 or another non-native ligand. The patient self-administers 0.1 mg/kg AZD-0328 or another non-native ligand orally daily for excess weight loss.

**Example 15. Treatment of a patient suffering from obesity**

**[0436]** In a non-limiting example, a patient suffering from obesity is treated using the compositions and methods disclosed herein. The patient is treated on Day One with $10^{13}$ vector genomes of AAV-SIM1 operably linked to a polynucleotide encoding any one of the engineered receptors disclosed herein in a volume of 1.0 mL delivered directly into the paraventricular nucleus (PVH) in the hypothalamus (i.e., intracranial, convection enhanced delivery). In this example, the AAV vector encodes the engineered channel under the control of the human Single-Minded Family BHLH Transcription Factor 1 (SIM1) promoter for selective neuronal expression in pro-opiomelanocortin (POMC) neurons and ultimately stimulation of the anorexigenic pathway. Two weeks post-injection, the patient returns to the clinic for a prescription for AZD-0328 or another non-native ligand. The patient self-administers 0.15 mg/kg AZD-0328 or another non-native ligand orally daily for excess weight loss (i.e. for apetitite suppression).

**Example 16. Treatment of a patient suffering from PTSD**

**[0437]** In a non-limiting example, a patient suffering from post-traumatic stress disorder (PTSD) is treated using the compositions and methods disclosed herein. The patient is treated on Day One with $10^{13}$ vector genomes of AAV-hSYN1 operably linked to a polynucleotide encoding any one of the engineered receptors disclosed herein in a volume of 1.0 mL delivered directly into the C6 stellate ganglion, (i.e., intraganglionic). In this example, the AAV vector encodes the engineered receptor under the control of the human Synapsin-1 (hSYN1) promoter for selective neuronal expression. Two weeks post-injection, the patient returns to the clinic for a prescription for AZD-0328 or another non-native ligand. The patient self-administers 0.15 mg/kg AZD-0328 or another non-native ligand orally daily for PTSD symptoms (i.e. for anxiety).

**Example 17. Treatment of a patient suffering from depression**

**[0438]** In a non-limiting example, a patient suffering from treatment-resistant depression (TRD) is treated using the compositions and methods disclosed herein. The patient is treated on Day One with $10^{13}$ vector genomes of AAV-hSYN1 operably linked to a polynucleotide encoding any one of the engineered receptors disclosed herein in a volume of 1.0 mL delivered directly into the vagus nerve, (i.e., intraneural). In this example, the AAV

vector encodes the engineered receptor under the control of the human Synapsin-1 (hSYN1) promoter for selective neuronal expression. Two weeks post-injection, the patient returns to the clinic for a prescription for AZD-0328 or another non-native ligand. The patient self-administers 0.1 mg/kg AZD-0328 or another non-native ligand orally daily for depression symptoms.

**Example 18. Treatment of a patient suffering from GERD**

**[0439]** In a non-limiting example, a patient suffering from gastroesophageal reflux disease (GERD) is treated using the compositions and methods disclosed herein. The patient is treated on Day One with $10^{13}$ vector genomes of AAV-hSYN1 operably linked to a polynucleotide encoding any one of the engineered receptors disclosed herein or AAV-CAG operably linked to a polynucleotide encoding any one of the engineered receptors disclosed herein in a volume of 1.0 mL delivered directly into the lower esophageal sphincter (LES) vagus nerve and myenteric plexus (i.e., intraneural) or smooth muscle (intramuscular), respectively. In this example, the AAV vector encodes the engineered receptor under the control of the human Synapsin-1 (hSYN1) promoter for selective neuronal expression or the CAG promoter for expression in LES myocytes. Two weeks post-injection, the patient returns to the clinic for a prescription for AZD-0328 or another non-native ligand. The patient self-administers 0.15 mg/kg AD-0328 or another non-native ligand orally daily for symptoms of GERD (i.e. acid reflux).

**Example 19. Treatment of a patient suffering from epilepsy**

**[0440]** In a non-limiting example, a patient suffering from seizures associated with epilepsy is treated using the compositions and methods disclosed herein. The patient is treated on Day One with $10^{13}$ vector genomes of AAV-CamKII$\alpha$ operably linked to a polynucleotide encoding any one of the engineered receptors disclosed herein in a volume of 1.0 mL delivered directly into a pre-determined seizure focus such as the motor cortex (i.e., intracranial). In this example, the AAV vector encodes the engineered receptor under the control of the human Calcium/calmodulin-dependent protein kinase II $\alpha$ (CamKII$\alpha$) promoter for selective neuronal expression in excitatory neurons. Two weeks post-injection, the patient returns to the clinic for a prescription for AZD-0328. The patient self-administers 0.1 mg/kg AZD-0328 orally daily for epileptic symptoms (i.e. seizures).

**Example 20. Treatment of a patient suffering from a movement disorder**

**[0441]** In a non-limiting example, a patient suffering from a movement disorder (*e.g.* Parkinsonian tremor) is treated using the compositions and methods disclosed herein. The patient is treated on Day One with $10^{13}$ vector genomes of AAV-CamKIIα operably linked to a polynucleotide encoding any one of the engineered receptors disclosed herein in a volume of 1.0 mL delivered directly into the subthalamic nucleus (i.e., intracranial STN). In this example, the AAV vector encodes the engineered receptor under the control of the human Calcium/calmodulin-dependent protein kinase II α (CamKIIα) promoter for selective neuronal expression in excitatory neurons. Two weeks post-injection, the patient returns to the clinic for a prescription forAZD-0328. The patient self-administers 0.1 mg/kg AZD-0328 orally daily for movement disorder symptoms (i.e. tremor).

## FURTHER NUMBERED EMBODIMENTS

**[0442]** Further embodiments of the instant invention are provided in the numbered embodiments below:

**[0443]** Embodiment 1. An engineered receptor, comprising a ligand binding domain derived from human α7 nicotinic acetylcholine receptor (α7-nAChR), wherein the ligand binding domain comprises an amino acid mutation at an amino acid residue corresponding to R101, Y115, L131, Q139, Y140, S172, Y210 or Y217 of SEQ ID NO: 4.

**[0444]** Embodiment 2. The engineered receptor of Embodiment 1, wherein the ligand binding domain comprises an amino acid sequence having at least 85% identity to amino acid residues 23-220 of SEQ ID NO: 4.

**[0445]** Embodiment 3. The engineered receptor of Embodiment 1 or 2, wherein the ligand binding domain comprises the amino acid mutation at two or more amino acid residues selected from those corresponding to R101, Y115, L131, Q139, Y140, S172, Y210 and Y217 of SEQ ID NO: 4.

**[0446]** Embodiment 4. The engineered receptor of any one of Embodiments 1-3, wherein the ligand binding domain comprises a mutation at the amino acid residue(s) corresponding to the indicated position of SEQ ID NO: 4:

a) Y140;

b) Y115 and L131;

c) Q139 and S172;

d) Q139 and Y210;

e) Q139, S172, and Y217;

f) Y115, Q139, and S172;

g) R101, L131, Q139, and Y217;

h) Y115 and S172;

i) L141, S172, and Y210; or

j) R101, L131, and Y217.

**[0447]** Embodiment 5. The engineered receptor of any one of Embodiments 1-4, wherein the mutation is amino acid substitution.

**[0448]** Embodiment 6. The engineered receptor of Embodiment 4, wherein the ligand binding domain comprises the amino acid substitutions(s) corresponding to the indicated position of SEQ ID NO: 4:

a) Y140R;

b) Y115E and L131M;

c) Q139W and S172D;

d) Q139W and Y210W;

e) Q139W, S172D, and Y217I;

f) Y115E, Q139E, and S172D;

g) R101F, L131G, Q139L, and Y217F;

h) Y115Q, and S172D;

i) L141M, S172D, and Y210W;

j) R101F, L131N, and Y217F;

k) L131G;

l) L131M;

m) Q139E;

n) Q139L;

o) Q139W; or

p) L141M.

**[0449]** Embodiment 7. The engineered receptor of any one of Embodiments 1-6, wherein the engineered receptor is a chimeric ligand gated ion channel (LGIC) receptor comprising an ion pore domain derived from a human Glycine receptor.

**[0450]** Embodiment 8. The engineered receptor of Embodiment 7, wherein the human Glycine receptor is human Glycine receptor α1, human Glycine receptor α2, or human Glycine receptor α3.

**[0451]** Embodiment 9. The engineered receptor of Embodiment 7, wherein the ion pore domain comprises an amino acid sequence having at least 85% identity to amino acids 255-

457 of SEQ ID NO: 2, 260-452 of SEQ ID NO: 83, amino acids 259-464 of SEQ ID NO: 85, or amino acids 259-449 of SEQ ID NO: 87.

**[0452]** Embodiment 10. The engineered receptor of any one of Embodiments 7-9, wherein the ligand binding domain of the engineered receptor comprises a Cys-loop domain derived from the human Glycine receptor.

**[0453]** Embodiment 11. The engineered receptor of Embodiment 10, wherein the Cys-loop domain comprises amino acids 166-172 of SEQ ID NO: 2.

**[0454]** Embodiment 12. The engineered receptor of Embodiment 10, wherein the Cys-loop domain comprises amino acids 166-180 of SEQ ID NO: 2.

**[0455]** Embodiment 13. The engineered receptor of any one of Embodiments 1-12, wherein the ligand binding domain of the engineered receptor comprises a β1-2 loop domain from the human Glycine receptor α1 subunit.

**[0456]** Embodiment 14. The engineered receptor of Embodiment 13, wherein the β1-2 loop domain comprises amino acids 81-84 of SEQ ID NO:2.

**[0457]** Embodiment 15. The engineered receptor of any one of Embodiments 1-14, wherein the engineered receptor comprises an amino acid sequence according to any one of SEQ ID NOs: 89-98.

**[0458]** Embodiment 16. An engineered chimeric ligand gated ion channel (LGIC) comprising a ligand binding domain from a first LGIC and an ion pore domain from a second LGIC, wherein the first LGIC is the human α7 nicotinic acetylcholine receptor (α7-nAChR) and comprises an amino acid mutation at an amino acid residue corresponding to R101, Y115, L131, Q139, Y140, S172, Y210 or Y217 of SEQ ID NO: 4.

**[0459]** Embodiment 17. The engineered chimeric LGIC of Embodiment 16, wherein the ligand binding domain comprises a mutation at the amino acid residue(s) corresponding to the indicated position of SEQ ID NO: 4:

a) Y140;

b) Y115 and L131;

c) Q139 and S172;

d) Q139 and Y210;

e) Q139, S172, and Y217;

f) Y115, Q139, and S172;

g) R101, L131, Q139, and Y217;

h) Y115 and S172;

i) L141, S172, and Y210; or

j) R101, L131, and Y217.

**[0460]** Embodiment 18. The engineered chimeric LGIC of Embodiment 16, wherein the ligand binding domain comprises the amino acid substitutions(s) corresponding to the indicated position of SEQ ID NO: 4:

a) Y140R;

b) Y115E and L131M;

c) Q139W and S172D;

d) Q139W and Y210W;

e) Q139W, S172D, and Y217I;

f) Y115E, Q139E, and S172D;

g) R101F, L131G, Q139L, and Y217F;

h) Y115Q, and S172D;

i) L141M, S172D, and Y210W;

j) R101F, L131N, and Y217F;

k) L131G;

l) L131M;

m) Q139E;

n) Q139L;

o) Q139W; or

p) L141M.

**[0461]** Embodiment 19. The engineered chimeric LGIC of any one of Embodiments 16-18, wherein the second LGIC is a human Glycine receptor.

**[0462]** Embodiment 20. The engineered chimeric LGIC of Embodiment 19, wherein the human Glycine receptor is human Glycine receptor α1.

**[0463]** Embodiment 21. The engineered chimeric LGIC of Embodiment 20, comprising a polypeptide sequence having at least 85% sequence identity to SEQ ID NO: 33.

**[0464]** Embodiment 22. The engineered receptor of any one of Embodiments 1-21, wherein the potency of the engineered receptor to acetylcholine is lower than the potency of the human α7 nicotinic acetylcholine receptor (α7-nAChR), or a control receptor, to acetylcholine.

**[0465]** Embodiment 23. The engineered receptor of Embodiment 22, wherein the potency of the engineered receptor to acetylcholine is at least 2-fold lower than the potency of

the human α7 nicotinic acetylcholine receptor (α7-nAChR), or a control receptor, to acetylcholine.

**[0466]** Embodiment 24. The engineered receptor of any one of Embodiments 1-23, wherein the potency of the engineered receptor to a non-native ligand is about the same as the potency of the human α7 nicotinic acetylcholine receptor (α7-nAChR), or a control receptor, to the non-native ligand.

**[0467]** Embodiment 25. The engineered receptor of any one of Embodiments 1-24, wherein the potency of the engineered receptor to a non-native ligand is higher than the potency of the human α7 nicotinic acetylcholine receptor (α7-nAChR), or a control receptor, to the non-native ligand.

**[0468]** Embodiment 26. The engineered receptor of Embodiment 25, wherein the potency of the engineered receptor to the non-native ligand is at least 2-fold higher than the potency of the human α7 nicotinic acetylcholine receptor (α7-nAChR), or a control receptor, to the non-native ligand.

**[0469]** Embodiment 27. The engineered receptor of any one of Embodiments 22-26, wherein the potency of the engineered receptor to a ligand is determined by the EC50 of the receptor for the ligand according to the YFP fluorescence quenching assay using Lenti-X 293T cells.

**[0470]** Embodiment 28. The engineered receptor of any one of Embodiments 1-27, wherein the efficacy of the engineered receptor in the presence of a non-native ligand is higher than the efficacy the human α7 nicotinic acetylcholine receptor (α7-nAChR), or a control receptor, in presence of the non-native ligand.

**[0471]** Embodiment 29. The engineered receptor of any one of Embodiments 1-28, wherein the efficacy of the engineered receptor in the presence of a non-native ligand is at least 2-fold higher than the efficacy the human α7 nicotinic acetylcholine receptor (α7-nAChR), or a control receptor, in presence of the non-native ligand.

**[0472]** Embodiment 30. The engineered receptor of any one of Embodiments 28-29, wherein determining the efficacy comprises determining the amount of current passed through the engineered receptor in vitro in the presence of the non-native ligand.

**[0473]** Embodiment 31. The engineered receptor of any one of Embodiments 24-30, wherein the non-native ligand is selected from the group consisting of AZD-0328, TC-6987, ABT-126, CNL002, TC-5619, CNL001, TC-6683, Varenicline, and Facinicline/RG3487.

**[0474]** Embodiment 32. The engineered receptor of Embodiment 31, wherein the non-native ligand is ABT-126.

**[0475]** Embodiment 33. The engineered receptor of Embodiment 31, wherein the non-native ligand is varenicline.

**[0476]** Embodiment 34. A polynucleotide encoding the engineered receptor of any one of Embodiments 1-33.

**[0477]** Embodiment 35. A polynucleotide encoding an engineered receptor comprising an amino acid sequence of any one of SEQ ID NOs: 89-98.

**[0478]** Embodiment 36. The polynucleotide of Embodiment 34 or 35, wherein the polynucleotide comprises a promoter operably linked to the nucleic acid encoding the engineered receptor.

**[0479]** Embodiment 37. The polynucleotide of Embodiment 36, wherein the promoter is a regulatable promoter.

**[0480]** Embodiment 38. The polynucleotide of Embodiment 37, wherein the regulatable promoter is active in an excitable cell.

**[0481]** Embodiment 39. The polynucleotide of Embodiment 38, wherein the excitable cell is a neuron or a myocyte.

**[0482]** Embodiment 40. The polynucleotide of Embodiment 39, wherein the excitable cell is a neuron.

**[0483]** Embodiment 41. A vector comprising the polynucleotide of any one of Embodiments 34-40.

**[0484]** Embodiment 42. The vector of Embodiment 41, wherein the vector is a plasmid, or a viral vector.

**[0485]** Embodiment 43. The vector of Embodiment 42, wherein the vector is a viral vector selected from the group consisting of an adenoviral vector, a retroviral vector, an adeno-associated viral (AAV) vector, and a herpes simplex-1 viral vector (HSV-1).

**[0486]** Embodiment 44. The vector of Embodiment 43, wherein the viral vector is an AVV vector, and wherein the AAV vector is AAV5 or a variant thereof, AAV6 or a variant thereof or AAV9 or a variant thereof; optionally a capsid protein of the AAV9 vector comprises a T492V mutation according to SEQ ID NO: 81.

**[0487]** Embodiment 45. A composition comprising the engineered receptor of any one of Embodiments 1-33, the polynucleotide of any one of Embodiments 34-40, or the vector of any one of Embodiments 41-44.

**[0488]** Embodiment 46. A pharmaceutical composition comprising the engineered receptor of any one of Embodiments 1-33, the polynucleotide of any one of Embodiments 34-40, or the vector of any one of Embodiments 41-44. and a pharmaceutically acceptable carrier.

**[0489]** Embodiment 47. A method of expressing an engineered receptor in a neuron, comprising contacting the neuron with the polynucleotide of any one of Embodiments 34-40, the vector of any one of Embodiments 41-44, the composition of Embodiment 45, or the pharmaceutical composition of Embodiment 46.

**[0490]** Embodiment 48. The method of Embodiment 47 or the polynucleotide of Embodiment 40, wherein the neuron is a neuron of the peripheral nervous system.

**[0491]** Embodiment 49. The method of Embodiment 47 or 48, or the polynucleotide of Embodiment 40, wherein the neuron is a neuron of the central nervous system.

**[0492]** Embodiment 50. The method of any one of Embodiments 47-49 or the polynucleotide of Embodiment 40, wherein the neuron is a nociceptive neuron.

**[0493]** Embodiment 51. The method of any one of Embodiments 47-50 or the polynucleotide of Embodiment 40, wherein the neuron is a non-nociceptive neuron.

**[0494]** Embodiment 52. The method of any one of Embodiments 47-51 or the polynucleotide of Embodiment 40, wherein the neuron is a hippocampal neuron, a dorsal root ganglion (DRG) neuron, a trigeminal ganglion (TG) neuron, a motor neuron, an excitatory neuron, an inhibitory neuron, or a sensory neuron.

**[0495]** Embodiment 53. The method of any one of Embodiments 47-52 or the polynucleotide of Embodiment 40, wherein the neuron is an Aδ afferent fiber, a C fiber or an Aβ afferent fiber.

**[0496]** Embodiment 54. The method of Embodiment 53 or the polynucleotide of Embodiment 40, wherein the neuron is Aβ afferent fiber.

**[0497]** Embodiment 55. The method of Embodiment 54 or the polynucleotide of Embodiment 40, wherein Aβ afferent fiber is an injured Aβ afferent fiber.

**[0498]** Embodiment 56. The method of Embodiment 54 or the polynucleotide of Embodiment 40, wherein Aβ afferent fiber is an uninjured Aβ afferent fiber.

**[0499]** Embodiment 57. The method of any one of Embodiments 47-56 or the polynucleotide of Embodiment 40, wherein the neuron expresses neurofilament 200 (NF200), piezo 2, and TLR-5.

**[0500]** Embodiment 58. The method of any one of Embodiments 47-57 or the polynucleotide of Embodiment 40, wherein the neuron does not express TrpV1, prostatic acid phosphatase, NaV1.1.

**[0501]** Embodiment 59. The method of any one of Embodiments 47-58, wherein the contacting step is performed in vitro, ex vivo, or in vivo.

**[0502]** Embodiment 60. The method of Embodiment 59, wherein the contacting step is performed in vivo in a subject.

**[0503]** Embodiment 61. The method of Embodiment 60, wherein the contacting step comprises administering the polynucleotide, the vector, the composition, or the pharmaceutical composition to the subject.

**[0504]** Embodiment 62. The method of Embodiment 61, wherein the contacting step is performed in vitro or ex vivo.

**[0505]** Embodiment 63. The method of Embodiment 62, wherein the contacting step comprises lipofection, nanoparticle delivery, particle bombardment, electroporation, sonication, or microinjection.

**[0506]** Embodiment 64. The method of any one of Embodiments 47-63, wherein the engineered receptor is capable of localizing to the cell surface of the neuron.

**[0507]** Embodiment 65. A method of inhibiting the activity of a neuron, comprising (a) contacting the neuron with the engineered receptor of any one of Embodiments 1-33, the polynucleotide of any one of Embodiments 34-40, or the vector of any one of Embodiments 41-44, the composition of Embodiment 45, or the pharmaceutical composition of Embodiment 46, and (b) contacting the neuron with a non-native ligand of the engineered receptor.

**[0508]** Embodiment 66. The method of Embodiment 65, wherein the neuron is a neuron of the peripheral nervous system.

**[0509]** Embodiment 67. The method of Embodiment 65, wherein the neuron is a neuron of the central nervous system.

**[0510]** Embodiment 68. The method of any of the Embodiments 65-67, wherein the neuron is a nociceptive neuron.

**[0511]** Embodiment 69. The method of any of the Embodiments 65-67, wherein the neuron is a non-nociceptive neuron.

**[0512]** Embodiment 70. The method of any one of Embodiments 65-69, wherein the neuron is a dorsal root ganglion (DRG) neuron, a trigeminal ganglion (TG) neuron, a motor neuron, an excitatory neuron, an inhibitory neuron, or a sensory neuron.

**[0513]** Embodiment 71. The method of any one of Embodiments 65-70, wherein the neuron is an Aδ afferent fiber, a C fiber or an Aβ afferent fiber.

**[0514]** Embodiment 72. The method of Embodiment 71, wherein the neuron is Aβ afferent fiber.

**[0515]** Embodiment 73. The method of Embodiment 72, wherein Aβ afferent fiber is an injured Aβ afferent fiber.

**[0516]** Embodiment 74. The method of Embodiment 72, wherein Aβ afferent fiber is an uninjured Aβ afferent fiber.

**[0517]** Embodiment 75. The method of any one of Embodiments 65-74, wherein the neuron expresses neurofilament 200 (NF200), piezo 2, and TLR-5.

**[0518]** Embodiment 76. The method of any one of Embodiments 65-75, wherein the neuron does not express TrpV1, prostatic acid phosphatase, NaV1.1.

**[0519]** Embodiment 77. The method of any one of Embodiments 65-76, wherein the contacting step (a) is performed in vitro, ex vivo, or in vivo.

**[0520]** Embodiment 78. The method of any one of Embodiments 65-77, wherein the contacting step (b) is performed in vitro, ex vivo, or in vivo.

**[0521]** Embodiment 79. The method of any one of Embodiments 65-78, wherein the contacting steps (a) and/or (b) are performed in vivo in a subject.

**[0522]** Embodiment 80. The method of Embodiment 79, wherein the contacting step (a) comprises administering the engineered receptor, the polynucleotide, the vector, or the pharmaceutical composition to the subject; and/or the contacting step (b) comprises administering the non-native ligand to the subject.

**[0523]** Embodiment 81. The method of any one of Embodiments 65-80, wherein the contacting step (a) and/or (b) comprises lipofection, nanoparticle delivery, particle bombardment, electroporation, sonication, or microinjection.

**[0524]** Embodiment 82. The method of any one of Embodiments 65-81, wherein the engineered receptor is capable of localizing to the cell surface of the neuron.

**[0525]** Embodiment 83. A method of treating and/or delaying the onset of a neurological disorder in a subject, in need thereof, comprising:

a. administering to the subject, a therapeutically effective amount of the engineered receptor of any one of Embodiments 1-33, the polynucleotide of any one of Embodiments 34-40, or the vector of any one of Embodiments 41-44, the composition of Embodiment 45, or the pharmaceutical composition of Embodiment 46, and

b. administering to the subject a non-native ligand of the engineered receptor.

**[0526]** Embodiment 84. The method of Embodiment 83, wherein the subject is administered the non-native ligand after step (a).

**[0527]** Embodiment 85. The method of Embodiment 83, wherein the subject is administered the non-native ligand concurrently with step (a).

**[0528]** Embodiment 86. The method of any one of Embodiments 83-85, wherein the neurological disorder is a seizure disorder, a movement disorder, an eating disorder, a spinal

cord injury, neurogenic bladder, allodynia, a spasticity disorder, pruritus, Alzheimer's disease, Parkinson's disease, post-traumatic stress disorder (PTSD), gastroesophageal reflux disease (GERD), addiction, anxiety, depression, memory loss, dementia, sleep apnea, stroke, narcolepsy, urinary incontinence, essential tremor, trigeminal neuralgia, burning mouth syndrome, or atrial fibrillation.

**[0529]** Embodiment 87. The method of Embodiment 86, wherein the neurological disorder is allodynia.

**[0530]** Embodiment 88. The method of any one of Embodiments 83-87, wherein the non-native ligand is selected from the group consisting of AZD-0328, ABT-126, TC6987, CNL002, TC-5619, CNL001, TC-6683, Varenicline, and Facinicline/RG3487.

**[0531]** Embodiment 89. The method of any one of Embodiments 83-88, wherein the non-native ligand is administered orally, subcutaneously, topically, or intravenously.

**[0532]** Embodiment 90. The method of Embodiment 89, wherein the non-native ligand is administered orally.

**[0533]** Embodiment 91. The method of any one of Embodiments 83-90, wherein the engineered receptor, the polynucleotide, the vector, the composition, or the pharmaceutical composition is administered subcutaneously, orally, intrathecally, topically, intravenously, intraganglioncally, intraneurally, intracranially, intraspinally, or to the cisterna magna.

**[0534]** Embodiment 92. The method of any one of Embodiments 83-91, wherein the engineered receptor, the polynucleotide, the vector, the composition, or the pharmaceutical composition is administered by transforaminal injection or intrathecally.

**[0535]** Embodiment 93. The method of any one of Embodiments 83-92, wherein the subject suffers from trigeminal neuralgia, and wherein the engineered receptor, the polynucleotide, the vector, the composition, or the pharmaceutical composition is administered to the trigeminal ganglion (TG) of the subject.

**[0536]** Embodiment 94. The method of any one of Embodiments 83-92, wherein the subject suffers from neuropathic pain, and wherein the engineered receptor, the polynucleotide, the vector, the composition, or the pharmaceutical composition is administered to the dorsal root ganglion (DRG) of the subject.

**[0537]** Embodiment 95. The method of any one of Embodiments 83-94, wherein the subject is a human.

**[0538]** Embodiment 96. The method of any one of Embodiments 83-95, wherein the therapeutically effectively amount diminishes the severity of a sign and/or or a symptom of the neurological disorder.

**[0539]** Embodiment 97. The method of any one of Embodiments 83-96, wherein the therapeutically effectively amount delays the onset of a sign and/or or a symptom of the neurological disorder.

**[0540]** Embodiment 98. The method of any one of Embodiments 83-97, wherein the therapeutically effectively amount eliminates a sign and/or or a symptom of the neurological disorder.

**[0541]** Embodiment 99. The method of any one of Embodiments 96-98, wherein the sign of the neurological disorder is nerve damage, nerve atrophy, and/or seizure.

**[0542]** Embodiment 100. The method of Embodiment 99, wherein the nerve damage is peripheral nerve damage.

**[0543]** Embodiment 101. The method of any one of Embodiments 96-100, wherein the symptom of the neurological disorder is pain.

**[0544]** Embodiment 102. A method of treating and/or delaying the onset of pain in a subject, in need thereof, comprising:

a. administering to the subject, a therapeutically effective amount of the engineered receptor of any one of Embodiments 1-33, the polynucleotide of any one of Embodiments 34-40, or the vector of any one of Embodiments 41-44, the composition of Embodiment 45, or the pharmaceutical composition of Embodiment 46, and

b. administering to the subject a non-native ligand of the engineered receptor.

**[0545]** Embodiment 103. The method of Embodiment 102, wherein the subject is administered the non-native ligand after step (a).

**[0546]** Embodiment 104. The method of Embodiment 102, wherein the subject is administered the non-native ligand concurrently with step (a).

**[0547]** Embodiment 105. The method of any one of Embodiments 102-104, wherein the non-native ligand is selected from the group consisting of AZD-0328, ABT-126, TC6987, CNL002, TC-5619, CNL001, TC-6683, Varenicline, and Facinicline/RG3487.

**[0548]** Embodiment 106. The method of any one of Embodiments 102-105, wherein the non-native ligand is administered orally, subcutaneously, topically, or intravenously.

**[0549]** Embodiment 107. The method of Embodiment 106, wherein the non-native ligand is administered orally.

**[0550]** Embodiment 108. The method of any one of Embodiments 102-107, wherein the engineered receptor, the polynucleotide, the vector, the composition, or the pharmaceutical composition is administered subcutaneously, orally, intrathecally, topically, intravenously, intraganglioncally, intraneurally, intracranially, intraspinally, or to the cisterna magna.

**[0551]** Embodiment 109. The method of any one of Embodiments 102-108, wherein the engineered receptor, the polynucleotide, the vector, the composition, or the pharmaceutical composition is administered by transforaminal injection or intrathecally.

**[0552]** Embodiment 110. The method of any one of Embodiments 102-109, wherein the subject suffers from trigeminal neuralgia, and wherein the engineered receptor, the polynucleotide, the vector, the composition, or the pharmaceutical composition is administered to the trigeminal ganglion (TG) of the subject.

**[0553]** Embodiment 111. The method of any one of Embodiments 102-110, wherein the subject suffers from neuropathic pain, and wherein the engineered receptor, the polynucleotide, the vector, the composition, or the pharmaceutical composition is administered to the dorsal root ganglion (DRG) of the subject.

**[0554]** Embodiment 112. The method of any one of Embodiments 102-111, wherein the subject is a human.

**[0555]** Embodiment 113. The method of any one of Embodiments 101-112, wherein the pain is neuropathic pain.

**[0556]** Embodiment 114. The method of any one of Embodiments 101-113, wherein the pain is associated with, caused by, or resulting from chemotherapy.

**[0557]** Embodiment 115. The method of any one of Embodiments 101-114, wherein the pain is associated with, caused by, or resulting from trauma.

**[0558]** Embodiment 116. The method of any of Embodiments 101-115, wherein the subject suffers from allodynia.

**[0559]** Embodiment 117. The method of any one of Embodiments 101-116, wherein the pain manifests after a medical procedure.

**[0560]** Embodiment 118. The method of any one of Embodiments 101-117, wherein the pain is associated with, is caused by, or resulting from childbirth or Caesarean section.

**[0561]** Embodiment 119. The method of any one of Embodiments 101-118, wherein the pain is associated with, is caused by, or resulting from migraine.

**[0562]** Embodiment 120. The method of any one of Embodiments 101-119, wherein the therapeutically effectively amount diminishes pain in the subject transiently, diminishes pain in the subject permanently, prevents the onset of pain in the subject, and/or eliminates pain in the subject.

**[0563]** Embodiment 121. The method of any one of Embodiments 101-120, wherein steps (a) and (b) are performed before the manifestation of pain in the subject.

**[0564]** Embodiment 122. A kit, comprising (a) the vector of any one of Embodiments 41-44, and (b) a non-native ligand of the engineered receptor.

**[0565]** Embodiment 123. A kit, comprising (a) the engineered receptor of any one of Embodiments 1-33, and (b) a non-native ligand of the engineered receptor.

**[0566]** Embodiment 124. The kit of Embodiment 122 or 123, wherein the engineered receptor and the non-native ligand is according to any one the combinations provided in Table 29.

**[0567]** Embodiment 125. The kit of Embodiment 122 or 124, comprising a device adapted to administration of the vector.

**[0568]** Embodiment 126. A method of treating focal epilepsy in a subject in need thereof, comprising administering an effective amount of the polynucleotide of any one of Embodiments 34-40 or the vector of any one of Embodiments 41-44.

**[0569]** Embodiment 127. The method of Embodiment 126, wherein the polynucleotide is delivered to a hippocampal neuron in the subject, or wherein the vector transduces the hippocampal neuron in the subject.

**[0570]** Embodiment 128. The method of Embodiment 126 or 127, wherein the vector is an AAV9 vector comprising a capsid polypeptide comprising or consisting of an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 81.

**[0571]** Embodiment 129. The method of any one of Embodiments 126-128, wherein the focal epilepsy is mesial temporal lobe epilepsy (mTLE).

**[0572]** Embodiment 130. The method of any one of Embodiments 126-129, wherein the polynucleotide or the vector is administered by intracranial administration, intrathecal (spine) administration, intrathecal (cisterna magna) administration, intracerebral administration, intraventricular administration, or direct injection into the epileptic focus in hippocampus.

**[0573]** Embodiment 131. A method of treating neuropathic pain in a subject in need thereof, comprising administering an effective amount of the polynucleotide of any one of Embodiments 34-40 or the vector of any one of Embodiments 41-44.

**[0574]** Embodiment 132. The method of Embodiment 131, wherein the neuropathic pain is peripheral neuropathy or trigeminal neuralgia.

**[0575]** Embodiment 133. The method of Embodiment 131 or 132, wherein the polynucleotide is delivered to a dorsal root ganglion neuron or a trigeminal ganglion neuron in the subject, or wherein the vector transduces the dorsal root ganglion neuron or the trigeminal ganglion neuron in the subject.

**[0576]** Embodiment 134. The method of any one of Embodiments 131 or 133, wherein the vector is an AAV9 vector comprising a capsid polypeptide comprising or consisting of an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 81 and a non-threonine mutation at the position corresponding to T492 of SEQ ID NO: 81.

**[0577]** Embodiment 135. The method of Embodiment 134, wherein the non-threonine mutation is a valine substitution.

**[0578]** Embodiment 136. The method of any one of Embodiments 131-135, wherein the polynucleotide or the vector is administered by intrathecal (IT) or intraganglionic (IG) administration.

**[0579]** Embodiment 137. The method of any one of Embodiments 126-136, further comprising administering a non-native ligand of the engineered receptor..

**[0580]** The preceding merely illustrates the principles of the disclosure. It will be appreciated that those skilled in the art will be able to devise various arrangements which, although not explicitly described or shown herein, embody the principles of the disclosure and are included within its spirit and scope. Furthermore, all examples and conditional language recited herein are principally intended to aid the reader in understanding the principles of the disclosure and the concepts contributed by the inventors to furthering the art, and are to be construed as being without limitation to such specifically recited examples and conditions. Moreover, all statements herein reciting principles, aspects, and embodiments of the disclosure as well as specific examples thereof, are intended to encompass both structural and functional equivalents thereof. Additionally, it is intended that such equivalents include both currently known equivalents and equivalents developed in the future, i.e., any elements developed that perform the same function, regardless of structure. The scope of the present disclosure, therefore, is not intended to be limited to the exemplary embodiments shown and described herein. Rather, the scope and spirit of the present disclosure is embodied by the appended claims.

# CLAIMS

**What is claimed is:**

1. An engineered receptor, comprising a ligand binding domain derived from human α7 nicotinic acetylcholine receptor (α7-nAChR), wherein the ligand binding domain comprises an amino acid mutation at an amino acid residue corresponding to R101, Y115, L131, Q139, Y140, S172, Y210 or Y217 of SEQ ID NO: 4.

2. The engineered receptor of claim 1, wherein the ligand binding domain comprises an amino acid sequence having at least 85% identity to amino acid residues 23-220 of SEQ ID NO: 4.

3. The engineered receptor of claim 1 or 2, wherein the ligand binding domain comprises the amino acid mutation at two or more amino acid residues selected from those corresponding to R101, Y115, L131, Q139, Y140, S172, Y210 and Y217 of SEQ ID NO: 4.

4. The engineered receptor of any one of claims 1-3, wherein the ligand binding domain comprises a mutation at the amino acid residue(s) corresponding to the indicated position of SEQ ID NO: 4:

    a) Y140;
    b) Y115 and L131;
    c) Q139 and S172;
    d) Q139 and Y210;
    e) Q139, S172, and Y217;
    f) Y115, Q139, and S172;
    g) R101, L131, Q139, and Y217;
    h) Y115 and S172;
    i) L141, S172, and Y210; or
    j) R101, L131, and Y217.

5. The engineered receptor of any one of claims 1-4, wherein the mutation is amino acid substitution.

6. The engineered receptor of claim 4, wherein the ligand binding domain comprises the amino acid substitutions(s) corresponding to the indicated position of SEQ ID NO: 4:

a) Y140R;
b) Y115E and L131M;
c) Q139W and S172D;
d) Q139W and Y210W;
e) Q139W, S172D, and Y217I;
f) Y115E, Q139E, and S172D;
g) R101F, L131G, Q139L, and Y217F;
h) Y115Q, and S172D;
i) L141M, S172D, and Y210W;
j) R101F, L131N, and Y217F;
k) L131G;
l) L131M;
m) Q139E;
n) Q139L;
o) Q139W; or
p) L141M.

7. The engineered receptor of any one of claims 1-6, wherein the engineered receptor is a chimeric ligand gated ion channel (LGIC) receptor comprising an ion pore domain derived from a human Glycine receptor.

8. The engineered receptor of claim 7, wherein the human Glycine receptor is human Glycine receptor α1, human Glycine receptor α2, or human Glycine receptor α3.

9. The engineered receptor of claim 7, wherein the ion pore domain comprises an amino acid sequence having at least 85% identity to amino acids 255-457 of SEQ ID NO: 2, 260-452 of SEQ ID NO: 83, amino acids 259-464 of SEQ ID NO: 85, or amino acids 259-449 of SEQ ID NO: 87.

10. The engineered receptor of any one of claims 7-9, wherein the ligand binding domain of the engineered receptor comprises a Cys-loop domain derived from the human Glycine receptor.

11. The engineered receptor of claim 10, wherein the Cys-loop domain comprises amino acids 166-172 of SEQ ID NO: 2.

12. The engineered receptor of claim 10, wherein the Cys-loop domain comprises amino acids 166-180 of SEQ ID NO: 2.

13. The engineered receptor of any one of claims 1-12, wherein the ligand binding domain of the engineered receptor comprises a β1-2 loop domain from the human Glycine receptor α1 subunit.

14. The engineered receptor of claim 13, wherein the β1-2 loop domain comprises amino acids 81-84 of SEQ ID NO:2.

15. The engineered receptor of any one of claims 1-14, wherein the engineered receptor comprises an amino acid sequence according to any one of SEQ ID NO: 89-98.

16. An engineered chimeric ligand gated ion channel (LGIC) comprising a ligand binding domain derived from a first LGIC and an ion pore domain derived from a second LGIC, wherein the first LGIC is the human α7 nicotinic acetylcholine receptor (α7-nAChR) and comprises an amino acid mutation at an amino acid residue corresponding to R101, Y115, L131, Q139, Y140, S172, Y210 or Y217 of SEQ ID NO: 4.

17. The engineered chimeric LGIC of claim 16, wherein the ligand binding domain comprises a mutation at the amino acid residue(s) corresponding to the indicated position of SEQ ID NO: 4:
    a) Y140;
    b) Y115 and L131;
    c) Q139 and S172;
    d) Q139 and Y210;
    e) Q139, S172, and Y217;
    f) Y115, Q139, and S172;
    g) R101, L131, Q139, and Y217;
    h) Y115 and S172;
    i) L141, S172, and Y210; or
    j) R101, L131, and Y217.

18. The engineered chimeric LGIC of claim 16, wherein the ligand binding domain comprises the amino acid substitutions(s) corresponding to the indicated position of SEQ ID NO: 4:
    a) Y140R;
    b) Y115E and L131M;
    c) Q139W and S172D;
    d) Q139W and Y210W;
    e) Q139W, S172D, and Y217I;
    f) Y115E, Q139E, and S172D;
    g) R101F, L131G, Q139L, and Y217F;
    h) Y115Q, and S172D;
    i) L141M, S172D, and Y210W;
    j) R101F, L131N, and Y217F;
    k) L131G;
    l) L131M;
    m) Q139E;
    n) Q139L;
    o) Q139W; or
    p) L141M.
19. The engineered chimeric LGIC of any one of claims 16-18, wherein the second LGIC is a human Glycine receptor.

20. The engineered chimeric LGIC of claim 19, wherein the human Glycine receptor is human Glycine receptor α1.

21. The engineered chimeric LGIC of claim 20, comprising a polypeptide sequence having at least 85% sequence identity to SEQ ID NO: 33.

22. The engineered receptor of any one of claims 1-21, wherein the potency of the engineered receptor to acetylcholine is lower than the potency of the human α7 nicotinic acetylcholine receptor (α7-nAChR), or a control receptor, to acetylcholine.

23. The engineered receptor of claim 22, wherein the potency of the engineered receptor to acetylcholine is at least 2-fold lower than the potency of the human α7 nicotinic acetylcholine receptor (α7-nAChR), or a control receptor, to acetylcholine.

24. The engineered receptor of any one of claims 1-23, wherein the potency of the engineered receptor to a non-native ligand is about the same as the potency of the human α7 nicotinic acetylcholine receptor (α7-nAChR), or a control receptor, to the non-native ligand.

25. The engineered receptor of any one of claims 1-24, wherein the potency of the engineered receptor to a non-native ligand is higher than the potency of the human α7 nicotinic acetylcholine receptor (α7-nAChR), or a control receptor, to the non-native ligand.

26. The engineered receptor of claim 25, wherein the potency of the engineered receptor to the non-native ligand is at least 2-fold higher than the potency of the human α7 nicotinic acetylcholine receptor (α7-nAChR), or a control receptor, to the non-native ligand.

27. The engineered receptor of any one of claims 22-26, wherein the potency of the engineered receptor to a ligand is determined by the EC50 of the receptor for the ligand according to the YFP fluorescence quenching assay using Lenti-X 293T cells.

28. The engineered receptor of any one of claims 1-27, wherein the efficacy of the engineered receptor in the presence of a non-native ligand is higher than the efficacy the human α7 nicotinic acetylcholine receptor (α7-nAChR), or a control receptor, in presence of the non-native ligand.

29. The engineered receptor of any one of claims 1-28, wherein the efficacy of the engineered receptor in the presence of a non-native ligand is at least 2-fold higher than the efficacy the human α7 nicotinic acetylcholine receptor (α7-nAChR), or a control receptor, in presence of the non-native ligand.

30. The engineered receptor of any one of claims 28-29, wherein determining the efficacy comprises determining the amount of current passed through the engineered receptor *in vitro* in the presence of the non-native ligand.

31. The engineered receptor of any one of claims 24-30, wherein the non-native ligand is selected from the group consisting of AZD-0328, TC-6987, ABT-126, CNL002, TC-5619, CNL001, TC-6683, Varenicline, and Facinicline/RG3487.

32. The engineered receptor of claim 31, wherein the non-native ligand is ABT-126.

33. The engineered receptor of claim 31, wherein the non-native ligand is varenicline.

34. A polynucleotide encoding the engineered receptor of any one of claims 1-33.

35. A polynucleotide encoding an engineered receptor comprising an amino acid sequence of any one of SEQ ID NOs: 89-98.

36. The polynucleotide of claim 34 or 35, wherein the polynucleotide comprises a promoter operably linked to the nucleic acid encoding the engineered receptor.

37. The polynucleotide of claim 36, wherein the promoter is a regulatable promoter.

38. The polynucleotide of claim 37, wherein the regulatable promoter is active in an excitable cell.

39. The polynucleotide of claim 38, wherein the excitable cell is a neuron or a myocyte.

40. The polynucleotide of claim 39, wherein the excitable cell is a neuron.

41. A vector comprising the polynucleotide of any one of claims 34-40.

42. The vector of claim 41, wherein the vector is a plasmid, or a viral vector.

43. The vector of claim 42, wherein the vector is a viral vector selected from the group consisting of an adenoviral vector, a retroviral vector, an adeno-associated viral (AAV) vector, and a herpes simplex-1 viral vector (HSV-1).

44. The vector of claim 43, wherein the viral vector is an AVV vector, and wherein the AAV vector is AAV5 or a variant thereof, AAV6 or a variant thereof or AAV9 or a variant thereof; optionally a capsid protein of the AAV9 vector comprises a T492V mutation according to SEQ ID NO: 81.

45. A composition comprising the engineered receptor of any one of claims 1-33, the polynucleotide of any one of claims 34-40, or the vector of any one of claims 41-44.

46. A pharmaceutical composition comprising the engineered receptor of any one of claims 1-33, the polynucleotide of any one of claims 34-40, or the vector of any one of claims 41-44. and a pharmaceutically acceptable carrier.

47. A method of expressing an engineered receptor in a neuron, comprising contacting the neuron with the polynucleotide of any one of claims 34-40, the vector of any one of claims 41-44, the composition of claim 45, or the pharmaceutical composition of claim 46.

48. The method of claim 47 or the polynucleotide of claim 40, wherein the neuron is a neuron of the peripheral nervous system.

49. The method of claim 47 or 48, or the polynucleotide of claim 40, wherein the neuron is a neuron of the central nervous system.

50. The method of any one of claims 47-49 or the polynucleotide of claim 40, wherein the neuron is a nociceptive neuron.

51. The method of any one of claims 47-50 or the polynucleotide of claim 40, wherein the neuron is a non-nociceptive neuron.

52. The method of any one of claims 47-51 or the polynucleotide of claim 40, wherein the neuron is a hippocampal neuron, a dorsal root ganglion (DRG) neuron, a trigeminal ganglion (TG) neuron, a motor neuron, an excitatory neuron, an inhibitory neuron, or a sensory neuron.

53. The method of any one of claims 47-52 or the polynucleotide of claim 40, wherein the neuron is an Aδ afferent fiber, a C fiber or an Aβ afferent fiber.

54. The method of claim 53 or the polynucleotide of claim 40, wherein the neuron is Aβ afferent fiber.

55. The method of claim 54 or the polynucleotide of claim 40, wherein Aβ afferent fiber is an injured Aβ afferent fiber.

56. The method of claim 54 or the polynucleotide of claim 40, wherein Aβ afferent fiber is an uninjured Aβ afferent fiber.

57. The method of any one of claims 47-56 or the polynucleotide of claim 40, wherein the neuron expresses neurofilament 200 (NF200), piezo 2, and TLR-5.

58. The method of any one of claims 47-57 or the polynucleotide of claim 40, wherein the neuron does not express TrpV1, prostatic acid phosphatase, NaV1.1.

59. The method of any one of claims 47-58, wherein the contacting step is performed *in vitro*, *ex vivo*, or *in vivo*.

60. The method of claim 59, wherein the contacting step is performed *in vivo* in a subject.

61. The method of claim 60, wherein the contacting step comprises administering the polynucleotide, the vector, the composition, or the pharmaceutical composition to the subject.

62. The method of claim 61, wherein the contacting step is performed *in vitro* or *ex vivo*.

63. The method of claim 62, wherein the contacting step comprises lipofection, nanoparticle delivery, particle bombardment, electroporation, sonication, or microinjection.

64. The method of any one of claims 47-63, wherein the engineered receptor is capable of localizing to the cell surface of the neuron.

65. A method of inhibiting the activity of a neuron, comprising (a) contacting the neuron with the engineered receptor of any one of claims 1-33, the polynucleotide of any one of claims 34-40, or the vector of any one of claims 41-44, the composition of claim 45, or the pharmaceutical composition of claim 46, and (b) contacting the neuron with a non-native ligand of the engineered receptor.

66. The method of claim 65, wherein the neuron is a neuron of the peripheral nervous system.

67. The method of claim 65, wherein the neuron is a neuron of the central nervous system.

68. The method of any of the claims 65-67, wherein the neuron is a nociceptive neuron.

69. The method of any of the claims 65-67, wherein the neuron is a non-nociceptive neuron.

70. The method of any one of claims 65-69, wherein the neuron is a dorsal root ganglion (DRG) neuron, a trigeminal ganglion (TG) neuron, a motor neuron, an excitatory neuron, an inhibitory neuron, or a sensory neuron.

71. The method of any one of claims 65-70, wherein the neuron is an Aδ afferent fiber, a C fiber or an Aβ afferent fiber.

72. The method of claim 71, wherein the neuron is Aβ afferent fiber.

73. The method of claim 72, wherein Aβ afferent fiber is an injured Aβ afferent fiber.

74. The method of claim 72, wherein Aβ afferent fiber is an uninjured Aβ afferent fiber.

75. The method of any one of claims 65-74, wherein the neuron expresses neurofilament 200 (NF200), piezo 2, and TLR-5.

76. The method of any one of claims 65-75, wherein the neuron does not express TrpV1, prostatic acid phosphatase, NaV1.1.

77. The method of any one of claims 65-76, wherein the contacting step (a) is performed *in vitro*, *ex vivo*, or *in vivo*.

78. The method of any one of claims 65-77, wherein the contacting step (b) is performed *in vitro*, *ex vivo*, or *in vivo*.

79. The method of any one of claims 65-78, wherein the contacting steps (a) and/or (b) are performed *in vivo* in a subject.

80. The method of claim 79, wherein the contacting step (a) comprises administering the engineered receptor, the polynucleotide, the vector, or the pharmaceutical composition to the subject; and/or the contacting step (b) comprises administering the non-native ligand to the subject.

81. The method of any one of claims 65-80, wherein the contacting step (a) and/or (b) comprises lipofection, nanoparticle delivery, particle bombardment, electroporation, sonication, or microinjection.

82. The method of any one of claims 65-81, wherein the engineered receptor is capable of localizing to the cell surface of the neuron.

83. A method of treating and/or delaying the onset of a neurological disorder in a subject, in need thereof, comprising:

a. administering to the subject, a therapeutically effective amount of the engineered receptor of any one of claims 1-33, the polynucleotide of any one of claims 34-40, or the vector of any one of claims 41-44, the composition of claim 45, or the pharmaceutical composition of claim 46, and
b. administering to the subject a non-native ligand of the engineered receptor.

84. The method of claim 83, wherein the subject is administered the non-native ligand after step (a).

85. The method of claim 83, wherein the subject is administered the non-native ligand concurrently with step (a).

86. The method of any one of claims 83-85, wherein the neurological disorder is a seizure disorder, a movement disorder, an eating disorder, a spinal cord injury, neurogenic bladder, allodynia, a spasticity disorder, pruritus, Alzheimer's disease, Parkinson's disease, post-traumatic stress disorder (PTSD), gastroesophageal reflux disease (GERD), addiction, anxiety, depression, memory loss, dementia, sleep apnea, stroke, narcolepsy, urinary incontinence, essential tremor, trigeminal neuralgia, burning mouth syndrome, or atrial fibrillation.

87. The method of claim 86, wherein the neurological disorder is allodynia.

88. The method of any one of claims 83-87, wherein the non-native ligand is selected from the group consisting of AZD-0328, ABT-126, TC6987, CNL002, TC-5619, CNL001, TC-6683, Varenicline, and Facinicline/RG3487.

89. The method of any one of claims 83-88, wherein the non-native ligand is administered orally, subcutaneously, topically, or intravenously.

90. The method of claim 89, wherein the non-native ligand is administered orally.

91. The method of any one of claims 83-90, wherein the engineered receptor, the polynucleotide, the vector, the composition, or the pharmaceutical composition is administered subcutaneously, orally, intrathecally, topically, intravenously, intraganglioncally, intraneurally, intracranially, intraspinally, or to the cisterna magna.

92. The method of any one of claims 83-91, wherein the engineered receptor, the polynucleotide, the vector, the composition, or the pharmaceutical composition is administered by transforaminal injection or intrathecally.

93. The method of any one of claims 83-92, wherein the subject suffers from trigeminal neuralgia, and wherein the engineered receptor, the polynucleotide, the vector, the composition, or the pharmaceutical composition is administered to the trigeminal ganglion (TG) of the subject.

94. The method of any one of claims 83-92, wherein the subject suffers from neuropathic pain, and wherein the engineered receptor, the polynucleotide, the vector, the composition, or the pharmaceutical composition is administered to the dorsal root ganglion (DRG) of the subject.

95. The method of any one of claims 83-94, wherein the subject is a human.

96. The method of any one of claims 83-95, wherein the therapeutically effectively amount diminishes the severity of a sign and/or or a symptom of the neurological disorder.

97. The method of any one of claims 83-96, wherein the therapeutically effectively amount delays the onset of a sign and/or or a symptom of the neurological disorder.

98. The method of any one of claims 83-97, wherein the therapeutically effectively amount eliminates a sign and/or or a symptom of the neurological disorder.

99. The method of any one of claims 96-98, wherein the sign of the neurological disorder is nerve damage, nerve atrophy, and/or seizure.

100. The method of claim 99, wherein the nerve damage is peripheral nerve damage.

101. The method of any one of claims 96-100, wherein the symptom of the neurological disorder is pain.

102. A method of treating and/or delaying the onset of pain in a subject, in need thereof, comprising:

a. administering to the subject, a therapeutically effective amount of the engineered receptor of any one of claims 1-33, the polynucleotide of any one of

claims 34-40, or the vector of any one of claims 41-44, the composition of claim 45, or the pharmaceutical composition of claim 46, and

b. administering to the subject a non-native ligand of the engineered receptor.

103. The method of claim 102, wherein the subject is administered the non-native ligand after step (a).

104. The method of claim 102, wherein the subject is administered the non-native ligand concurrently with step (a).

105. The method of any one of claims 102-104, wherein the non-native ligand is selected from the group consisting of AZD-0328, ABT-126, TC6987, CNL002, TC-5619, CNL001, TC-6683, Varenicline, and Facinicline/RG3487.

106. The method of any one of claims 102-105, wherein the non-native ligand is administered orally, subcutaneously, topically, or intravenously.

107. The method of claim 106, wherein the non-native ligand is administered orally.

108. The method of any one of claims 102-107, wherein the engineered receptor, the polynucleotide, the vector, the composition, or the pharmaceutical composition is administered subcutaneously, orally, intrathecally, topically, intravenously, intraganglioncally, intraneurally, intracranially, intraspinally, or to the cisterna magna.

109. The method of any one of claims 102-108, wherein the engineered receptor, the polynucleotide, the vector, the composition, or the pharmaceutical composition is administered by transforaminal injection or intrathecally.

110. The method of any one of claims 102-109, wherein the subject suffers from trigeminal neuralgia, and wherein the engineered receptor, the polynucleotide, the vector, the composition, or the pharmaceutical composition is administered to the trigeminal ganglion (TG) of the subject.

111. The method of any one of claims 102-110, wherein the subject suffers from neuropathic pain, and wherein the engineered receptor, the polynucleotide, the vector, the composition, or the pharmaceutical composition is administered to the dorsal root ganglion (DRG) of the subject.

112. The method of any one of claims 102-111, wherein the subject is a human.

113. The method of any one of claims 101-112, wherein the pain is neuropathic pain.

114. The method of any one of claims 101-113, wherein the pain is associated with, caused by, or resulting from chemotherapy.

115. The method of any one of claims 101-114, wherein the pain is associated with, caused by, or resulting from trauma.

116. The method of any of claims 101-115, wherein the subject suffers from allodynia.

117. The method of any one of claims 101-116, wherein the pain manifests after a medical procedure.

118. The method of any one of claims 101-117, wherein the pain is associated with, is caused by, or resulting from childbirth or Caesarean section.

119. The method of any one of claims 101-118, wherein the pain is associated with, is caused by, or resulting from migraine.

120. The method of any one of claims 101-119, wherein the therapeutically effectively amount diminishes pain in the subject transiently, diminishes pain in the subject permanently, prevents the onset of pain in the subject, and/or eliminates pain in the subject.

121. The method of any one of claims 101-120, wherein steps (a) and (b) are performed before the manifestation of pain in the subject.

122. A kit, comprising (a) the vector of any one of claims 41-44, and (b) a non-native ligand of the engineered receptor.

123. A kit, comprising (a) the engineered receptor of any one of claims 1-33, and (b) a non-native ligand of the engineered receptor.

124. The kit of claim 122 or 123, wherein the engineered receptor and the non-native ligand is according to any one the combinations provided in Table 29.

125. The kit of claim 122 or 124, comprising a device adapted to administration of the vector.

126. A method of treating focal epilepsy in a subject in need thereof, comprising administering an effective amount of the polynucleotide of any one of claims 34-40 or the vector of any one of claims 41-44.

127. The method of claim 126, wherein the polynucleotide is delivered to a hippocampal neuron in the subject, or wherein the vector transduces the hippocampal neuron in the subject.

128. The method of claim 126 or 127, wherein the vector is an AAV9 vector comprising a capsid polypeptide comprising or consisting of an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 81.

129. The method of any one of claims 126-128, wherein the focal epilepsy is mesial temporal lobe epilepsy (mTLE).

130. The method of any one of claims 126-129, wherein the polynucleotide or the vector is administered by intracranial administration, intrathecal (spine) administration, intrathecal (cisterna magna) administration, intracerebral administration, intraventricular administration, or direct injection into the epileptic focus in hippocampus.

131. A method of treating neuropathic pain in a subject in need thereof, comprising administering an effective amount of the polynucleotide of any one of claims 34-40 or the vector of any one of claims 41-44.

132. The method of claim 131, wherein the neuropathic pain is peripheral neuropathy or trigeminal neuralgia.

133. The method of claim 131 or 132, wherein the polynucleotide is delivered to a dorsal root ganglion neuron or a trigeminal ganglion neuron in the subject, or wherein the vector transduces the dorsal root ganglion neuron or the trigeminal ganglion neuron in the subject.

134. The method of any one of claims 131 or 133, wherein the vector is an AAV9 vector comprising a capsid polypeptide comprising or consisting of an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 81 and a non-threonine mutation at the position corresponding to T492 of SEQ ID NO: 81.

135. The method of claim 134, wherein the non-threonine mutation is a valine substitution.

136. The method of any one of claims 131-135, wherein the polynucleotide or the vector is administered by intrathecal (IT) or intraganglionic (IG) administration.

137. The method of any one of claims 126-136, further comprising administering a non-native ligand of the engineered receptor.

# FIG. 1A

| Name | Amino Acid Mutation(s) in SEQ ID NO: 33 | Acetylcholine | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 10 uM ach | 30 uM ach | 50 uM ach | 100 uM ach | 300 uM ach | 500 uM ach | 1 mM ach | 3 mM ach | 5 mM ach | 10 mM ach |
| CODA71 | - | 15.61 | 63.31 | 73.16 | 74.94 | 65.94 | 62.78 | 74.24 | 76.27 | 77.15 | 77.96 |
| CODA75 | SEQ ID NO: 29 | -12.88 | -18.67 | -0.29 | -14.46 | -16.31 | -17.41 | -16.46 | -12.38 | -20.88 | -8.69 |
| CODA217 | W77F | -5.86 | | | 70.06 | | | 68.71 | 73.45 | | |
| CODA109 | S170T | 82.91 | 82.53 | 82.11 | 83.68 | | | 69.89 | 78.12 | | |
| CODA236 | R101F | -18.24 | | | 60 | | | 66.07 | 71.38 | | |
| CODA237 | R101M | -16.79 | | | -18.63 | | | 46.28 | 69.31 | | |
| CODA238 | R101W | -16.58 | | | 75.18 | | | 78.99 | 75.74 | | |
| CODA282 | Y115D | 6.81 | | | 1.19 | | | -14.05 | 2.37 | | |
| CODA283 | Y115E_N379K | -6.16 | | | -11.12 | | | -2.74 | 53.61 | | |
| CODA325 | L131G | 5.66 | | | 74.07 | | | 62.65 | 69.49 | | |
| CODA326 | L131A | 7.43 | | | 73.57 | | | 71.8 | 74.49 | | |
| CODA330 | L131F | 11.07 | | | 43.34 | | | 77.52 | 74.89 | | |
| CODA333 | L131S | -10.11 | | | -10.05 | | | 32.11 | 57.37 | | |
| CODA334 | L131Q | -2.63 | 30.06 | | 71.27 | | | 79.66 | 74.9 | | |
| CODA335 | L131T | -9.13 | | | -9.29 | | | 0.57 | 35.37 | | |
| CODA339 | L131D | 4.56 | | | 10.19 | | | 77.34 | 77.79 | | |
| CODA340 | L131E | 15.29 | 66.56 | | 81.31 | | | 80.77 | 77.31 | | |
| CODA342 | L131R | -2.57 | | | -3.18 | | | 78.98 | 75.75 | | |
| CODA337 | L131N | 28.32 | | | 81.8 | | | 71.22 | 73.7 | | |
| CODA945 | Q139E | -1.25 | | | 71.21 | | | 71.79 | 76.04 | | |
| CODA377 | S172D | -3.19 | | | 0.07 | | | 71.7 | 76.95 | | |
| CODA405 | Y210V | -6.31 | | | -14.48 | | | -2.82 | 1.06 | | |
| CODA407 | Y210F | -3.75 | | | -1.6 | | | 71.65 | 79.09 | | |
| CODA409 | Y210W | -5.43 | | | 17.14 | | | 78.84 | 78.55 | | |

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**FIG. 1A (continued)**

| Name | Amino Acid Mutation(s) in SEQ ID NO: 33 | Acetylcholine | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 10 uM ach | 30 uM ach | 50 uM ach | 100 uM ach | 300 uM ach | 500 uM ach | 1 mM ach | 3 mM ach | 5 mM ach | 10 mM ach |
| CODA534 | L131S_S172D | -11.3 | | | -27.53 | | | 2.84 | 14.65 | | |
| CODA535 | L131T_S172D | -12.24 | | | -26.23 | | | -1.16 | 2.97 | | |
| CODA536 | L131D_S172D | -12.91 | | | -20.88 | | | 79.85 | 79.98 | | |
| CODA805 | Y115D_S170T | -0.46 | | | -4.93 | | | -11.18 | -3.53 | | |
| CODA806 | Y115D_L131Q | 9.47 | | | 6.12 | | | -1.56 | 17.57 | | |
| CODA807 | Y115D_L131E | 15.45 | | | 13.77 | | | 27.42 | 53.62 | | |
| CODA952 | Y140I | -1.97 | | | 2.79 | | | 58.81 | 66.9 | | |
| CODA965 | Y140C | -21.71 | | | 3.49 | | | 63.34 | 51.92 | | |
| CODA1025 | R101F_L131G | 12.57 | | -3.46 | 12.65 | -6.96 | -6.77 | 19.42 | 65.06 | | |
| CODA1027 | R101F_L131D | -1.62 | | -1.6 | -2.62 | -6.12 | -7.71 | -5.73 | 33.69 | | |
| CODA1039 | Y115E_Y210W | -2.97 | | -3.94 | 0.35 | -4.13 | -5.18 | 1.21 | 26.69 | | |
| CODA1045 | R101W_Y210V | -2.52 | | | -11.98 | -16.71 | -12.87 | -14.41 | -7.4 | | -1.5 |
| CODA1047 | R101F_Y210V | -4.26 | | | -13.05 | -11.73 | -10.75 | -8.81 | -6.77 | | -1.03 |
| CODA1048 | R101F_Y210F | | | | -17.31 | -13.7 | -10.1 | 5.53 | 68.61 | | 78.22 |
| CODA1053 | R101M_L131A | -3.98 | | | -11 | -7.92 | 7.36 | 57.28 | 60.54 | | 79.46 |
| CODA1054 | R101M_L131F | -3.98 | | | -10.34 | -10.25 | -11.46 | 1.77 | 36.81 | | 74.02 |
| CODA1055 | R101W_Y115E_Y210W | | | | -15.63 | -12.05 | -12.87 | -11.55 | 12.68 | | 37.58 |
| CODA1056 | R101F_Y115E_Y210W | | | | -20.55 | -19.32 | -19.75 | -14.08 | 14.07 | | 25.7 |
| CODA1138 | W77F_R101F_L131D | -12.92 | -15.36 | | -19.35 | -14.79 | | 37.5 | 61.73 | | |
| CODA1140 | R101F_L131N_S172D | -20.07 | -16.92 | | -25.8 | -11.21 | | 9.02 | 45 | | |
| CODA1157 | Q139E_S172D | -36.89 | -37.78 | | -25.67 | -18.94 | | 16.38 | 45.71 | | |
| CODA1173 | S172D_Y210W | | | | | | | -6.12 | 11.31 | | |

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# FIG. 1B

| Name | Amino Acid Mutation(s) in SEQ ID NO: 33 | CNL001 | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1nM CNL001 | 1nM CNL001 | 10nM CNL001 | 10nM CNL001 | 50nM CNL001 | 50nM CNL001 | 100nM CNL001 | 100nM CNL001 | 1uM CNL001 | 1uM CNL001 |
| CODA71 | - | 3.727 | 20.231 | 81.096 | 82.368 | 83.62 | 84.482 | 84.297 | 85.368 | 85.647 | 86.358 |
| CODA75 | SEQ ID NO: 29 | -8.496 | -2.266 | 1.816 | -4.649 | -5.922 | -5.597 | -8.159 | -5.058 | -4.272 | -1.281 |
| CODA217 | W77F | -22.273 | -19.415 | -9.563 | 6.398 | 61.938 | 66.837 | 79.791 | 80.908 | 83.444 | 83.905 |
| CODA109 | S170T | 19.755 | 65.484 | 79.024 | 81.563 | 81.926 | 83.092 | 83 | 83.811 | 81.834 | 83.858 |
| CODA236 | R101F | 9.048 | 26.601 | 71.533 | 72.948 | 75.895 | 74.238 | 76 | 75.861 | 76.367 | 76.162 |
| CODA237 | R101M | -2.114 | -5.079 | 15.096 | 50.554 | 66.585 | 74.462 | 78 | 77.411 | 79.06 | 79.162 |
| CODA238 | R101W | 32.42 | 54.768 | 75.149 | 75.339 | 75.881 | 77.844 | 78 | 78.575 | 81.126 | 79.482 |
| CODA282 | Y115D | -15.837 | -6.519 | -4.862 | -8.244 | -3.807 | -2.871 | -7.924 | 2.11 | 11.047 | 8.04 |
| CODA283 | Y115E_N379K | -0.713 | -0.621 | 7.739 | 15.277 | 19.489 | 33.703 | 56.886 | 60.171 | 69.836 | 73.855 |
| CODA325 | L131G | 74.429 | 78.715 | 79.033 | 79.322 | 79.966 | 80.091 | 81 | 79.701 | 79.289 | 80.581 |
| CODA326 | L131A | 76.424 | 80.567 | 80.284 | 81.232 | 81.575 | 82.544 | 81 | 81.718 | 82.334 | 81.539 |
| CODA330 | L131F | 76.289 | 78.896 | 79.339 | 80.245 | 80.831 | 80.605 | 81 | 80.663 | 81.293 | 81.428 |
| CODA333 | L131S | 76.165 | 78.463 | 79.349 | 78.238 | 80.221 | 81.789 | 81.855 | 80.766 | 81.707 | 82.665 |
| CODA334 | L131Q | 76.165 | 78.463 | 79.349 | 78.238 | 80.221 | 81.789 | 82 | 80.766 | 81.707 | 82.665 |
| CODA335 | L131T | -9.942 | -0.475 | 17.889 | 38.46 | 61.741 | 69.159 | 73.635 | 76.092 | 80.633 | 79.637 |
| CODA339 | L131D | 73.711 | 76.44 | 77.049 | 77.76 | 77.882 | 78.708 | 79.567 | 79.273 | 79.992 | 79.927 |
| CODA340 | L131E | 76.413 | 77.144 | 78.258 | 78.763 | 79.757 | 79.327 | 79.011 | 79.948 | 79.855 | 80.618 |
| CODA342 | L131R | 23.532 | 26.054 | 43.69 | 54.016 | 59.955 | 56.091 | 64.536 | 58.987 | 65.987 | 64.312 |
| CODA337 | L131N | 76.917 | 79.554 | 79.479 | 79.506 | 81.465 | 81.297 | 82.619 | 81.722 | 81.96 | 81.773 |
| CODA945 | Q139E | 6.65 | 13.512 | 78.678 | 82.723 | 84.91 | 84.849 | 85.046 | 84.642 | 85.493 | 86.071 |
| CODA377 | S172D | 67.544 | 74.209 | 74.346 | 75.816 | 77.068 | 76.99 | 79.352 | 77.865 | 80.272 | 79.432 |
| CODA405 | Y210V | -16.246 | -4.217 | -6.142 | -10.003 | -11.054 | -10.06 | -3.832 | -3.784 | 1.129 | 7.1 |
| CODA407 | Y210F | -10.845 | -2.629 | 4.66 | 12.246 | 29.307 | 41.78 | 50.654 | 62.054 | 81.742 | 81.304 |
| CODA409 | Y210W | -8.914 | -14.319 | -1.444 | 4.393 | 11.897 | 11.434 | 12.719 | 16.053 | 14.077 | 15.495 |

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**FIG. 1B (continued)**

| Name | Amino Acid Mutation(s) in SEQ ID NO: 33 | CNL001 | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1nM CNL001 | 1nM CNL001 | 10nM CNL001 | 10nM CNL001 | 50nM CNL001 | 50nM CNL001 | 100nM CNL001 | 100nM CNL001 | 1uM CNL001 | 1uM CNL001 |
| CODA534 | L131S_S172D | 23.438 | 28.59 | 56.882 | 56.777 | 65.044 | 67.373 | 68.666 | 70.357 | 73.019 | 73.527 |
| CODA535 | L131T_S172D | 8.736 | 24.572 | 60.552 | 63.09 | 73.242 | 73.889 | 76.147 | 76.21 | 78.988 | 78.818 |
| CODA805 | Y115D_S170T | -10.729 | -15.278 | -20.403 | -14.905 | -14.303 | -15.741 | -12.637 | -10.606 | -13.535 | -11.335 |
| CODA806 | Y115D_L131Q | 6.587 | -5.179 | -2.366 | -1.272 | 2.763 | 1.995 | 3.091 | 1.45 | 7.331 | 9.103 |
| CODA807 | Y115D_L131E | 0.885 | -1.707 | -0.567 | 0.043 | 5.141 | 9.409 | 9.796 | 11.009 | 27.914 | 29.109 |
| CODA952 | Y140I | 0.368 | -1.976 | -3.923 | 4.348 | 74.107 | 75.866 | 80.323 | 81.996 | 83.489 | 82.208 |
| CODA965 | Y140C | -12.765 | -12.608 | 68.134 | 71.394 | 80.298 | 82.159 | 82.404 | 82.14 | 82.34 | 80.649 |
| CODA1025 | R101F_L131G | -18.527 | -21.577 | 14.063 | 49.818 | 77.765 | 79.23 | 80.544 | 80.695 | 81.191 | 82.385 |
| CODA1027 | R101F_L131D | 24.049 | 36.755 | 52.927 | 61.449 | 67.308 | 68.77 | 69.049 | 71.613 | 71.811 | 69.907 |
| CODA1039 | Y115E_Y210W | -1.12 | -3.941 | 6.275 | 11.943 | 12.949 | 18.635 | 17.576 | 18.319 | 19.329 | 20.672 |
| CODA1045 | R101W_Y210V | 0.804 | 11.275 | 15.843 | 16.619 | 15.205 | 18.771 | 9.332 | 18.218 | 35.294 | 36.542 |
| CODA1047 | R101F_Y210V | -7.82 | -8.382 | -5.381 | -1.431 | -0.999 | 3.909 | -0.823 | -5.885 | 1.638 | 3.483 |
| CODA1048 | R101F_Y210F | 9.114 | 0.845 | 28.944 | 53.629 | 68.581 | 74.379 | 80.541 | 80.774 | 83.143 | 81.751 |
| CODA1053 | R101M_L131A | -8.554 | -13.711 | 39.631 | 66.809 | 82.122 | 82.031 | 83.312 | 83.986 | 84.363 | 83.877 |
| CODA1054 | R101M_L131F | 21.932 | 39.689 | 54.192 | 64.111 | 71.409 | 70.989 | 74.148 | 71.722 | 71.378 | 71.411 |
| CODA1055 | R101W_Y115E_Y210W | -12.889 | -13.295 | -10.401 | -7.981 | -1.972 | -1.661 | 0.277 | 4.325 | 2.88 | 3.259 |
| CODA1056 | R101F_Y115E_Y210W | -8.104 | -12.153 | -10.462 | -4.735 | -8.617 | -1.606 | -1.243 | 1.838 | -0.567 | 4.663 |
| CODA1138 | W77F_R101F_L131D | -6.175 | 5.89 | -2.032 | 7.601 | 9.263 | 4.423 | 24 | 30.448 | 73.823 | 73.271 |
| CODA1140 | R101F_L131N_S172D | 52.592 | 61.248 | 71.678 | 71.881 | 73.807 | 75.649 | 76 | 76.329 | 76.168 | 74.076 |
| CODA1157 | Q139E_S172D | 68.362 | 69.495 | 75.078 | 74.193 | 77.481 | 77.205 | 77 | 77.042 | 78.2 | 76.244 |
| CODA1173 | S172D_Y210W | 16.321 | 30.264 | 61.978 | 65.343 | 73.006 | 74.903 | 76 | 76.759 | 76.928 | 76.619 |

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**FIG. 1C**

| Name | Amino Acid Mutation(s) in SEQ ID NO: 33 | TC-6683 | | | | |
|---|---|---|---|---|---|---|
| | | 30nM TC-6683 | 300nM TC-6683 | 3uM TC-6683 | 30uM TC-6683 | 100uM TC-6683 |
| CODA71 | - | -14.43 | -13.08 | -13.71 | -14.02 | -8.77 |
| CODA282 | Y115D | 0.365 | -6.865 | -4.927 | -11.259 | -11.894 |
| CODA325 | L131G | -21.27 | -18.33 | -7.03 | 56.84 | 64.51 |
| CODA326 | L131A | -16.28 | -11.73 | -2.38 | -9.77 | 0.97 |
| CODA330 | L131F | -22.26 | -18.89 | -9.45 | -9.23 | -10.75 |
| CODA333 | L131S | -23.88 | -21.46 | -21.07 | -16.15 | -18.96 |
| CODA334 | L131Q | -17.99 | -23.26 | -18.84 | -16.92 | -15.59 |
| CODA335 | L131T | -13.84 | -20.66 | -17.86 | -18.91 | -16.88 |
| CODA339 | L131D | -18.32 | -17.25 | -10 | -8.51 | -9.77 |
| CODA340 | L131E | 30.14 | 33.02 | 41.68 | 35.75 | 24.32 |
| CODA342 | L131R | -16.26 | -22.45 | -13.48 | -12.68 | -10.33 |
| CODA337 | L131N | -11.51 | -13.17 | -5.46 | 8.53 | 14.41 |
| CODA945 | Q139E | -26.47 | -18.62 | -13.39 | -16.6 | -12.89 |
| CODA377 | S172D | -20.23 | -13.94 | -14.17 | 1.86 | 43.61 |
| CODA534 | L131S_S172D | -22.464 | -23.976 | -24.897 | -20.474 | -22.253 |
| CODA535 | L131T_S172D | -29.362 | -28.954 | -23.708 | -23.431 | -18.324 |
| CODA536 | L131D_S172D | -22.285 | -23.926 | -20.514 | -3.078 | 7.37 |
| CODA807 | Y115D_L131E | -26.801 | -25.363 | -23.651 | -26.235 | -24.182 |
| CODA952 | Y140I | -27.7 | -26.87 | -31.2 | -33.12 | -24.67 |
| CODA965 | Y140C | -8.21 | -16.37 | -19.48 | -24.32 | -24.99 |
| CODA1025 | R101F_L131G | -1.645 | 3.043 | 0.082 | 2.449 | 10.437 |
| CODA1027 | R101F_L131D | 1.189 | 0.939 | 1.327 | -0.845 | -3.136 |
| CODA1039 | Y115E_Y210W | -1.608 | -3.491 | -6.153 | -9.263 | -9.19 |
| CODA1045 | R101W_Y210V | 1.735 | 0.642 | 2.403 | 6.006 | -4.277 |
| CODA1047 | R101F_Y210V | -24.195 | -24.234 | -21.291 | -16.363 | -24.9 |
| CODA1048 | R101F_Y210F | -29.055 | -33.935 | -24.613 | -21.752 | -22.815 |
| CODA1054 | R101M_L131F | -22.996 | -27.055 | -18.611 | -13.879 | -21.521 |
| CODA1055 | R101W_Y115E_Y210W | -30.056 | -30.801 | -25.558 | -18.295 | -17.554 |
| CODA1056 | R101F_Y115E_Y210W | -19.889 | -22.443 | -12.623 | -10.728 | -10.959 |

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**FIG. 1D**

| | | TC-5619/Bradanicline | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Name** | **Amino Acid Mutation(s) in SEQ ID NO: 33** | **1 nM brd** | **3 nM brd** | **10 nM brd** | **30 nM brd** | **100 nM brd** | **300 nM brd** | **1 uM brd** |
| CODA71 | - | 9.41 | 44.38 | 76.61 | 70.23 | 78.62 | 75.51 | |
| CODA75 | SEQ ID NO: 29 | -13.39 | -15.78 | -13.47 | -16.41 | | -17.56 | |
| CODA217 | W77F | | -12.1 | | -14.72 | | -6.3 | |
| CODA109 | S170T | | 70.58 | | 80.71 | | 80.66 | |
| CODA236 | R101F | | 49.33 | | 77.38 | | 77.51 | |
| CODA237 | R101M | | -14.87 | | 69.93 | | 76.57 | |
| CODA238 | R101W | | 74.7 | | 76.78 | | 77.01 | |
| CODA282 | Y115D | -0.54 | -7.22 | -2.82 | -16.67 | -0.02 | -12.37 | 31.23 |
| CODA283 | Y115E_N379K | 0.59 | -6.41 | -1.58 | -14.24 | 33.36 | 43.81 | 75.39 |
| CODA325 | L131G | | 68.45 | | 76.95 | | 77.1 | |
| CODA326 | L131A | | 72.24 | | 77.4 | | 78.02 | |
| CODA330 | L131F | -12.4 | 25.12 | 72.19 | 76.35 | 78.94 | 77.06 | 78.61 |
| CODA333 | L131S | -0.85 | -2.05 | -2.8 | -2.07 | 30.34 | 50.56 | 72.42 |
| CODA334 | L131Q | | 41.92 | | 63.65 | | 64.85 | |
| CODA335 | L131T | -2.39 | -4.08 | -3.3 | -9.69 | -1.05 | 10.53 | 34.72 |
| CODA339 | L131D | 4.12 | 19.15 | 68.98 | 77.29 | 81.41 | 75.79 | 82.26 |
| CODA340 | L131E | | 68.57 | | 76.85 | | 78.49 | |
| CODA342 | L131R | -7.83 | -12.89 | -10.47 | 59.48 | 70.19 | 75.11 | 77.42 |
| CODA337 | L131N | | 71.02 | | 78.43 | | 77.12 | |
| CODA945 | Q139E | | 14.15 | | 78.18 | | 76.8 | |
| CODA377 | S172D | 0.77 | -4.59 | 0.06 | -10.48 | 0.25 | -10.61 | 3.82 |
| CODA405 | Y210V | | 9.92 | | 4.37 | | 59 | |
| CODA407 | Y210F | | -0.53 | | 8.71 | | 76.26 | |
| CODA409 | Y210W | -13.45 | -0.64 | 37.3 | 76.36 | 72.72 | 75.47 | 78.73 |

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**FIG. 1D (continued)**

| | | TC-5619/Bradanicline | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Name** | **Amino Acid Mutation(s) in SEQ ID NO: 33** | **1 nM brd** | **3 nM brd** | **10 nM brd** | **30 nM brd** | **100 nM brd** | **300 nM brd** | **1 uM brd** |
| CODA534 | L131S_S172D | -1.13 | -3.83 | -3.78 | | -2.03 | | 1.68 |
| CODA536 | L131D_S172D | -1.24 | 0.25 | -2.57 | 0.78 | 4.51 | 30.97 | 44.41 |
| CODA805 | Y115D_S170T | -9.78 | -12.06 | -9.02 | -16.3 | -7.44 | -10.93 | -4.55 |
| CODA806 | Y115D_L131Q | 0.92 | -4.65 | 0.38 | -8.96 | 12.18 | -0.3 | 45.47 |
| CODA807 | Y115D_L131E | -1.52 | -5.35 | -3.49 | -2.16 | 4.18 | 70.2 | 43.11 |
| CODA952 | Y140I | | -2.45 | | 66.97 | | 78.48 | |
| CODA965 | Y140C | | -18.49 | | 22.66 | | 38.02 | |
| CODA1025 | R101F_L131G | -10.61 | 2.63 | 17.71 | 68.48 | | 75.12 | |
| CODA1027 | R101F_L131D | -10.07 | -0.8 | 1.74 | 53.95 | | 76.74 | |
| CODA1039 | Y115E_Y210W | -5.55 | 6.78 | -2.7 | 34.43 | | 70.05 | |
| CODA1045 | R101W_Y210V | -1.431 | -14.993 | -5.52 | 15.49 | 72.105 | 71.411 | |
| CODA1047 | R101F_Y210V | -5.548 | -9.432 | -3.068 | 2.368 | 47.529 | 74.413 | |
| CODA1048 | R101F_Y210F | -2.084 | -9.098 | -2.587 | 55.818 | 76.957 | 74.268 | |
| CODA1053 | R101M_L131A | 0.008 | 43.21 | 77.672 | 76.277 | 76.856 | 75.32 | |
| CODA1054 | R101M_L131F | 6.609 | -7.635 | 4.459 | 69.782 | 77.633 | 72.933 | |
| CODA1055 | R101W_Y115E_Y210W | -8.113 | -11.136 | 38.755 | 39.111 | 61.378 | 52.398 | |
| CODA1056 | R101F_Y115E_Y210W | -7.894 | -11.812 | 36.211 | 40.624 | 65.869 | 59.661 | |

**FIG. 1E**

| | | CNL002 | | | | | |
|---|---|---|---|---|---|---|---|
| **Name** | **Amino Acid Mutation(s) in SEQ ID NO: 33** | **1nM CNL002** | **10nM CNL002** | **50nM CNL002** | **100 nM CNL002** | **1 uM CNL002** | **30 uM CNL002** |
| CODA71 | - | | | | 49.61 | 74.15 | 75.21 |
| CODA75 | SEQ ID NO: 29 | | | | -7.94 | -9.81 | -15.27 |
| CODA217 | W77F | | | | 67.81 | 69.42 | 73.64 |
| CODA109 | S170T | | | | 73.48 | 70.03 | |
| CODA236 | R101F | | | | -9.56 | 56.9 | 70.28 |
| CODA237 | R101M | | | | -8.68 | -2.24 | 66.99 |
| CODA238 | R101W | | | | 2.95 | 67.93 | 73.02 |
| CODA282 | Y115D | | | | -9.7 | -8.37 | 3.4 |
| CODA283 | Y115E_N379K | | | | -5.81 | 9.69 | -12.85 |
| CODA325 | L131G | | | | 45.62 | 74.72 | 77.4 |
| CODA326 | L131A | | | | 27.88 | 73.79 | 75.89 |
| CODA330 | L131F | | | | -3.76 | 68.51 | 77.77 |
| CODA333 | L131S | | | | -15.44 | 15.21 | |
| CODA334 | L131Q | | | | 49.9 | 80.72 | 80.97 |
| CODA335 | L131T | | | | -14.53 | 6.45 | 57.43 |
| CODA339 | L131D | | | | 12.83 | 77.73 | 80.58 |
| CODA340 | L131E | | | | 49.97 | 67.8 | 64.58 |
| CODA342 | L131R | | | | -17.2 | 8.12 | 57.24 |
| CODA337 | L131N | | | | 76.37 | 80.18 | 80.93 |
| CODA945 | Q139E | | | | 60.5 | 75.41 | 77.46 |
| CODA377 | S172D | | | | 59.35 | 79.26 | 79.96 |
| CODA405 | Y210V | | | | -14.3 | -7.56 | -2.02 |
| CODA407 | Y210F | | | | -12.47 | -3.93 | 80.97 |
| CODA409 | Y210W | | | | -14.92 | -0.85 | 29.62 |

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**FIG. 1E (continued)**

| Name | Amino Acid Mutation(s) in SEQ ID NO: 33 | CNL002 1nM CNL002 | CNL002 10nM CNL002 | CNL002 50nM CNL002 | 100 nM CNL002 | 1 uM CNL002 | 30 uM CNL002 |
|---|---|---|---|---|---|---|---|
| CODA534 | L131S_S172D | | | | 14.26 | 45.55 | 64.93 |
| CODA535 | L131T_S172D | | | | 1.23 | 35.77 | 68.29 |
| CODA536 | L131D_S172D | | | | 71.72 | 77.26 | 77.5 |
| CODA805 | Y115D_S170T | | | | -18.17 | -15.2 | -14 |
| CODA806 | Y115D_L131Q | | | | -10.03 | -3.33 | 6.96 |
| CODA807 | Y115D_L131E | | | | -5.71 | 13.03 | 25.2 |
| CODA952 | Y140I | | | | 32.58 | 69.08 | 73.28 |
| CODA965 | Y140C | | | | 43.24 | 42.72 | 51.43 |
| CODA1025 | R101F_L131G | | | | 12.48 | 46.04 | 39.82 |
| CODA1027 | R101F_L131D | | | | 7.75 | 61.97 | 65.77 |
| CODA1039 | Y115E_Y210W | | | | 21.85 | 47.37 | 59.97 |
| CODA1138 | W77F_R101F_L131D | -2.209 | 43.044 | 73.418 | 81.66 | 81.69 | |
| CODA1140 | R101F_L131N_S172D | 0.337 | 59.894 | 76.501 | 79.03 | 82.85 | |
| CODA1157 | Q139E_S172D | | | | 80.62 | 81.32 | |
| CODA1173 | S172D_Y210W | | | | 70.15 | 77.33 | |

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# FIG. 1F

| | | ABT-126 | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Name** | **Amino Acid Mutation(s) in SEQ ID NO: 33** | **50 nM abt** | **100 nM abt** | **300 nM abt** | **500 nM abt** | **1 uM abt** | **3 uM abt** | **10 uM abt** | **30 uM abt** | **50 uM abt** | **100 uM abt** |
| CODA71 | - | -1.98 | 24.27 | 56.8 | 76.28 | 73.16 | 70.98 | 72.62 | 77.49 | 77.96 | 77.81 |
| CODA75 | SEQ ID NO: 29 | -17.07 | -16.76 | -16.76 | -14.68 | -16 | -14.11 | -13.98 | -21.05 | -21.35 | -24.12 |
| CODA217 | W77F | 37.81 | 57.31 | 12.61 | | 66.05 | | | | | |
| CODA109 | S170T | 55.83 | 68.89 | 72.52 | | 70.96 | | | | | |
| CODA236 | R101F | -18.69 | -23.01 | -20.92 | | 4.99 | | | | | |
| CODA237 | R101M | -24.34 | -20.22 | -20.57 | | -19.55 | | | | | |
| CODA238 | R101W | -14.97 | -14.66 | -16.35 | | 50.02 | | | | | |
| CODA282 | Y115D | -21.22 | -19.7 | -15.55 | | -14.31 | | | | | |
| CODA283 | Y115E_N379K | -22.65 | -20.55 | -12.93 | | 1.71 | | | | | |
| CODA325 | L131G | -17.39 | 6.89 | 38.65 | | 45.82 | | | | | |
| CODA326 | L131A | -21.91 | -0.5 | 34.86 | | 56.66 | | | | | |
| CODA330 | L131F | -16.22 | -11.89 | 39.23 | | 66.5 | | | | | |
| CODA333 | L131S | -20.99 | -16.21 | -13.34 | | 4.96 | | | | | |
| CODA334 | L131Q | 23.65 | 61.83 | 76.44 | | 77.68 | | | | | |
| CODA335 | L131T | -18 | -16.39 | -15.05 | | -5.56 | | | | | |
| CODA339 | L131D | -16.65 | -12.3 | 18.5 | | 59.09 | | | | | |
| CODA340 | L131E | 2.2 | 25.39 | 36.48 | | 50.76 | | | | | |
| CODA342 | L131R | -18.14 | -18.14 | -0.81 | 23.29 | 45.76 | 53.03 | 64.08 | 71.98 | 73.98 | 73.51 |
| CODA337 | L131N | -11.64 | 34.79 | 60.08 | | 65.99 | | | | | |
| CODA945 | Q139E | -8.27 | 4.76 | 30.88 | | 60.66 | | | | | |
| CODA377 | S172D | -1.53 | 18.18 | 59.15 | | 72.28 | | | | | |
| CODA405 | Y210V | -2.9 | -2.06 | -0.19 | | -3.64 | | | | | |
| CODA407 | Y210F | -13.07 | 0.1 | 5.97 | | 50.23 | | | | | |
| CODA409 | Y210W | 8.23 | 55.53 | 74.82 | 77.39 | 77.77 | 69.33 | 74.79 | 75.61 | 75.72 | 78.52 |

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## FIG. 1F (continued)

| Name | Amino Acid Mutation(s) in SEQ ID NO: 33 | ABT-126 | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 50 nM abt | 100 nM abt | 300 nM abt | 500 nM abt | 1 uM abt | 3 uM abt | 10 uM abt | 30 uM abt | 50 uM abt | 100 uM abt |
| CODA534 | L131S_S172D | -11.96 | -10.72 | 11.65 | | -3.06 | | | | | |
| CODA535 | L131T_S172D | -11.09 | -10.37 | -4 | | 4.05 | | | | | |
| CODA536 | L131D_S172D | -3.19 | 7.58 | -1.65 | | 31.29 | | | | | |
| CODA805 | Y115D_S170T | -11.83 | -11.5 | -8.01 | | -9.34 | | | | | |
| CODA806 | Y115D_L131Q | -1.81 | -2.61 | -3.78 | | -2.6 | | | | | |
| CODA807 | Y115D_L131E | -1.68 | -2.25 | -2.37 | | 3.68 | | | | | |
| CODA952 | Y140I | -12.34 | -11.57 | -5.21 | | 3.93 | | | | | |
| CODA965 | Y140C | -10.75 | -4.11 | 18.93 | | 52.64 | | | | | |
| CODA1025 | R101F_L131G | -11.53 | -7.61 | 6.55 | | 25.08 | | | | | |
| CODA1027 | R101F_L131D | -11.89 | -16.07 | -11.21 | | -9.38 | | | | | |
| CODA1039 | Y115E_Y210W | -17.32 | -13.93 | 9.9 | 36.97 | 48.37 | 50.5 | 58.13 | 64.1 | 66.67 | 68.63 |
| CODA1045 | R101W_Y210V | -24.16 | -24.7 | -16.09 | | -11.61 | | | | | |
| CODA1047 | R101F_Y210V | -14.1 | -11.72 | -12.98 | | -15.09 | | | | | |
| CODA1048 | R101F_Y210F | -23.28 | -21.78 | -19.35 | | -16.33 | | | | | |
| CODA1053 | R101M_L131A | -27.01 | -18.76 | -17.8 | | -6.55 | | | | | |
| CODA1054 | R101M_L131F | -19.54 | -18.28 | -16.87 | | -11.77 | | | | | |
| CODA1055 | R101W_Y115E_Y210W | -21.36 | -22.28 | -14.63 | | -7.34 | | | | | |
| CODA1056 | R101F_Y115E_Y210W | -20.96 | -21.95 | -10.96 | | 10.79 | | | | | |
| CODA1138 | W77F_R101F_L131D | -11.71 | -5.13 | 15.09 | | 56.63 | | 75.93 | | | |
| CODA1140 | R101F_L131N_S172D | -7.87 | -5.35 | -3.67 | | 3.64 | | 5.91 | | | |
| CODA1157 | Q139E_S172D | -26.77 | -24.64 | -10.64 | | 19.57 | | 55.64 | | | |
| CODA1173 | S172D_Y210W | 5.15 | 25.08 | 66.86 | | 66.94 | | 78.67 | | | |

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FIG. 1G

| Name | Amino Acid Mutation(s) in SEQ ID NO: 33 | AZD-0328 | | | | |
|---|---|---|---|---|---|---|
| | | 100 nM azd0328 | 300 nM azd0328 | 1 uM azd0328 | 10 uM azd0328 | 30 uM azd0328 |
| CODA71 | - | 73.59 | 78.09 | | | 79.05 |
| CODA75 | SEQ ID NO: 29 | -8.83 | -11.15 | | | -11.92 |
| CODA217 | W77F | -7.57 | 7.08 | | | 81.59 |
| CODA109 | S170T | 84.01 | 87.08 | | | 87.27 |
| CODA236 | R101F | -9.78 | 49.64 | | | 81.14 |
| CODA237 | R101M | -17.84 | -11.98 | | | 81.2 |
| CODA238 | R101W | 22.94 | 75.38 | | | 81.8 |
| CODA282 | Y115D | 3.19 | 4.79 | | | 76.78 |
| CODA283 | Y115E_N379K | -2.1 | 38.2 | | | 79.44 |
| CODA325 | L131G | 79.75 | 80.62 | | | 80.19 |
| CODA326 | L131A | 80.39 | 82.07 | | | 82.23 |
| CODA330 | L131F | 27.56 | 73.35 | | | 77.14 |
| CODA333 | L131S | 38.49 | 67.13 | | | 80.61 |
| CODA334 | L131Q | 80.35 | 83.15 | | | 82.53 |
| CODA335 | L131T | 11.33 | 44.15 | | | 80.96 |
| CODA339 | L131D | 62.87 | 77.03 | | | 78.63 |
| CODA340 | L131E | 82.48 | 82.04 | | | 81.8 |
| CODA342 | L131R | 1.79 | 66.58 | | | 80.55 |
| CODA337 | L131N | 82.1 | 82.52 | | | 82.32 |
| CODA945 | Q139E | -5.79 | 58.47 | | | 66.57 |
| CODA377 | S172D | 65.04 | 81.73 | | | 82.3 |
| CODA405 | Y210V | -8.13 | -3.35 | | | -2.86 |
| CODA407 | Y210F | 0.85 | 1.25 | | | 82.08 |
| CODA409 | Y210W | -6.57 | -4.84 | | | 81.64 |

**FIG. 1G (continued)**

| Name | Amino Acid Mutation(s) in SEQ ID NO: 33 | AZD-0328 | | | | |
|---|---|---|---|---|---|---|
| | | 100 nM azd0328 | 300 nM azd0328 | 1 uM azd0328 | 10 uM azd0328 | 30 uM azd0328 |
| CODA534 | L131S_S172D | -9.32 | 4.94 | | | 60.61 |
| CODA535 | L131T_S172D | -7.02 | -0.48 | | | 70.16 |
| CODA536 | L131D_S172D | 51.44 | 77.12 | | | 79.26 |
| CODA805 | Y115D_S170T | -0.71 | -1.73 | | | 47.39 |
| CODA806 | Y115D_L131Q | 12.15 | 30.92 | | | 68.65 |
| CODA807 | Y115D_L131E | 24.49 | 45.34 | | | 71.21 |
| CODA952 | Y140I | -12.96 | -13.21 | | | 68.5 |
| CODA965 | Y140C | -16.69 | 1.3 | | | 21.78 |
| CODA1025 | R101F_L131G | 6.26 | 37.98 | | | 79.31 |
| CODA1027 | R101F_L131D | -4.76 | -7.52 | | | 74.9 |
| CODA1039 | Y115E_Y210W | -10.45 | -13 | | | 30.76 |

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# FIG. 1H

| Name | Amino Acid Mutation(s) in SEQ ID NO: 33 | Facinicline | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 30 nM fac | 50 nM fac | 100 nM fac | 300 nM fac | 1 uM fac | 10 uM fac | 30 uM fac |
| CODA71 | - | 53.42 | | 83.6 | 77.44 | | | 79.62 |
| CODA75 | SEQ ID NO: 29 | -11.68 | | 4.35 | -10.55 | | | -5.25 |
| CODA217 | W77F | -10.14 | | | 68.29 | | | 87.35 |
| CODA109 | S170T | 76.45 | | 82.45 | 71.09 | | | 69.41 |
| CODA236 | R101F | -28.54 | | | 72.98 | | | 87.6 |
| CODA237 | R101M | -24.14 | | | -14.38 | | | 88.78 |
| CODA238 | R101W | -19.61 | | | 76.54 | | | 87.94 |
| CODA282 | Y115D | 10.17 | | 24.77 | 51.04 | | 79.76 | 81.7 |
| CODA283 | Y115E_N379K | 6.39 | | | 78.36 | | 81.93 | 78.63 |
| CODA325 | L131G | 75.75 | | | 78.01 | | | 76.61 |
| CODA326 | L131A | 69.94 | | | 80.07 | | | 78.32 |
| CODA330 | L131F | 2.9 | | | 74.96 | | 81.97 | 78.31 |
| CODA333 | L131S | 6.27 | | | 69.8 | | 80.07 | 81.35 |
| CODA334 | L131Q | 83.07 | | 85.36 | 84.19 | | | 82.75 |
| CODA335 | L131T | 1.24 | | | 46.92 | | 79.06 | 81.06 |
| CODA339 | L131D | 12.67 | | | 80.4 | | 82.18 | 82.14 |
| CODA340 | L131E | 82.51 | | 83.6 | 82.92 | | | 82.68 |
| CODA342 | L131R | -6.2 | | | 76.75 | | 82.16 | 81.71 |
| CODA337 | L131N | 82.25 | | | 83.59 | | | 82.82 |
| CODA945 | Q139E | -7.16 | | | 71.17 | | | 72.14 |
| CODA377 | S172D | 4.93 | | | 84.13 | | 66.96 | 85.29 |
| CODA405 | Y210V | -26.25 | | | -23.52 | | | 0.83 |
| CODA407 | Y210F | -21.94 | | | -22.46 | | | 82.63 |
| CODA409 | Y210W | -10.93 | | | -5.82 | | 60.97 | 57.77 |

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**FIG. 1H (continued)**

| Name | Amino Acid Mutation(s) in SEQ ID NO: 33 | Facinicline | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 30 nM fac | 50 nM fac | 100 nM fac | 300 nM fac | 1 uM fac | 10 uM fac | 30 uM fac |
| CODA534 | L131S_S172D | -23.44 | | | 2.75 | | 69.71 | 61.44 |
| CODA535 | L131T_S172D | -21.36 | | | 4.17 | | | 65.94 |
| CODA536 | L131D_S172D | 24.15 | | | 80.17 | | 81.34 | 79.9 |
| CODA805 | Y115D_S170T | 38.08 | | -14.37 | 19.41 | | 67.33 | 79.67 |
| CODA806 | Y115D_L131Q | 41.32 | | 59.51 | 74.86 | | 78.53 | 84.45 |
| CODA807 | Y115D_L131E | 45.76 | | 63.92 | 79.43 | | 80.1 | 84.91 |
| CODA952 | Y140I | -8.83 | | | 51.6 | | | 71.01 |
| CODA965 | Y140C | -12.29 | | | 20.11 | | | 37.62 |
| CODA1025 | R101F_L131G | -8.99 | | | 10.21 | | | 78.24 |
| CODA1027 | R101F_L131D | -10.05 | | | -12.54 | | | 70.96 |
| CODA1039 | Y115E_Y210W | -13.46 | | | 5.43 | | | 49.06 |

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**FIG. 1I**

| Name | Amino Acid Mutation(s) in SEQ ID NO: 33 | TC-6987 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 uM tc6987 | 3 uM tc6987 | 5 uM tc6987 | 10 uM tc6987 | 30 uM tc6987 | 50 uM tc6987 | 100 uM tc6987 | 300 uM tc6987 | 500 uM tc6987 |
| CODA71 | - | 31.56 | 31.82 | 30.75 | 56.68 | 51.46 | 61.26 | 43.21 | 7.79 | 51.58 |
| CODA75 | SEQ ID NO: 29 | -11.79 | | | -14.31 | | | | | -19.65 |
| CODA217 | W77F | -10.35 | | | -13.93 | | | | | -7.54 |
| CODA109 | S170T | 71.45 | | | 71.04 | | | | | 66.2 |
| CODA236 | R101F | 45.34 | | | 70.04 | | | | | 75.86 |
| CODA237 | R101M | -21.62 | | | 21.52 | | | | | 60.33 |
| CODA238 | R101W | 72.47 | | | 72.04 | | | | | 76.56 |
| CODA282 | Y115D | 13.01 | | | 14.73 | | | | | 14.29 |
| CODA283 | Y115E_N379K | -9.54 | | | -7.06 | | | | | -15.15 |
| CODA325 | L131G | 64.59 | | | 76.28 | | | | | 70.95 |
| CODA326 | L131A | 73.37 | | | 78.18 | | | | | 75.42 |
| CODA330 | L131F | 53.37 | | | 74.23 | | | | | 72.25 |
| CODA333 | L131S | -3.87 | -20.2 | -15.25 | 4.92 | 14.75 | 21.33 | 29.7 | 20.79 | 44.37 |
| CODA334 | L131Q | 74.1 | | | 81.7 | | | | | 80.18 |
| CODA335 | L131T | -0.14 | -8.32 | -2.13 | 0.13 | -6.86 | -9.5 | -10.98 | -12.16 | -2.41 |
| CODA339 | L131D | 50 | 80.39 | 79.93 | 80.99 | 83.11 | 83.18 | 82.16 | 64.58 | 76.77 |
| CODA340 | L131E | 80.13 | | | 81.29 | | | | | 79.6 |
| CODA342 | L131R | 2.51 | | | 46.22 | | | | | 68.33 |
| CODA337 | L131N | 79.06 | | | 82.36 | | | | | 80.19 |
| CODA945 | Q139E | -0.97 | | | 21.53 | | | | | 7.39 |
| CODA377 | S172D | -9.74 | -14.04 | -0.95 | -7.41 | -16.12 | -12.79 | -11.77 | -19.68 | -17.02 |
| CODA405 | Y210V | -22.63 | | | -23.03 | | | | | -40.88 |
| CODA407 | Y210F | -15.22 | | | -19.89 | | | | | -33.32 |
| CODA409 | Y210W | 62.82 | | | 78.66 | | | | | 70.35 |

**FIG. 1I (continued)**

| Name | Amino Acid Mutation(s) in SEQ ID NO: 33 | TC-6987 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 uM tc6987 | 3 uM tc6987 | 5 uM tc6987 | 10 uM tc6987 | 30 uM tc6987 | 50 uM tc6987 | 100 uM tc6987 | 300 uM tc6987 | 500 uM tc6987 |
| CODA534 | L131S_S172D | -20.99 | | | -27.62 | | | | | -24.22 |
| CODA535 | L131T_S172D | -21.8 | | | -27.08 | | | | | -21.56 |
| CODA536 | L131D_S172D | -18.24 | | | -23.06 | | | | | -19.3 |
| CODA805 | Y115D_S170T | 31.9 | | | 25.54 | | | | | 21.13 |
| CODA806 | Y115D_L131Q | 38.83 | | | 31.59 | | | | | 29.83 |
| CODA807 | Y115D_L131E | 36.07 | | | 31.35 | | | | | 28.18 |
| CODA952 | Y140I | 6.03 | | | 2.25 | | | | | -0.24 |
| CODA965 | Y140C | 2.73 | | | 30.37 | | | | | 33.07 |
| CODA1025 | R101F_L131G | -13.32 | | | 43.71 | | | | | 53.98 |
| CODA1027 | R101F_L131D | 0.88 | | | 56.27 | | | | | 71.3 |
| CODA1039 | Y115E_Y210W | 7.26 | | | 11 | | | | | 7.97 |

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**FIG. 1J**

| Name | Amino Acid Mutation(s) in SEQ ID NO: 33 | Varenicline | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3nM var | 3nM var | 30nM var | 30nM var | 300nM var | 300nM var | 500nM var | 500nM var | 1uM var | 1uM var |
| CODA71 | - | -2.36 | -3.522 | -4.255 | -3.636 | 46.195 | 45.522 | 69.253 | 71.705 | 79.254 | 79.244 |
| CODA75 | SEQ ID NO: 29 | 4.161 | -6.216 | -5.173 | -6.593 | -5.733 | -7.312 | -10.35 | -7.586 | -5.637 | -3.757 |
| CODA217 | W77F | 5.662 | 7.57 | 13.968 | 19.079 | 16.183 | 18.025 | 23.366 | 19.206 | 34.69 | 30.504 |
| CODA109 | S170T | -20.96 | -19.953 | -16.094 | -21.502 | 64.373 | 71.198 | 71.892 | 73.53 | 75.461 | 78.578 |
| CODA236 | R101F | 19.949 | 22.334 | 15.764 | 16.213 | 23.135 | 22.987 | 35.048 | 34.188 | 71.031 | 71.005 |
| CODA237 | R101M | 18.429 | 16.83 | 23.07 | 15.868 | 18.819 | 19.155 | 19.117 | 22.593 | 24.525 | 23.383 |
| CODA238 | R101W | 17.896 | 12.564 | 16.263 | 22.117 | 77.492 | 79.056 | 80.186 | 80.065 | 81.263 | 80.702 |
| CODA282 | Y115D | 2.111 | -12.265 | -4.433 | -6.153 | -0.861 | -1.308 | 0.733 | -4.633 | -4.512 | 0.02 |
| CODA325 | L131G | -12.241 | -9.415 | 75.334 | 72.278 | 78.305 | 77.83 | 75.936 | 76.788 | 77.805 | 77.352 |
| CODA326 | L131A | -5.939 | -2.294 | 68.663 | 70.718 | 79.88 | 79.742 | 79.311 | 80.029 | 80.952 | 79.329 |
| CODA330 | L131F | -3.016 | -1.452 | -0.193 | -1.853 | 6.915 | 6.513 | 27.593 | 29.863 | 67.499 | 65.57 |
| CODA333 | L131S | -4.782 | 1.048 | 2.084 | 5.022 | 12.162 | 12.979 | 29.238 | 25.614 | 43.863 | 39.346 |
| CODA334 | L131Q | -5.192 | -4.327 | -0.688 | 0.672 | 75.189 | 74.923 | 79.048 | 80.337 | 79.766 | 76.836 |
| CODA335 | L131T | 0.398 | 3.947 | 5.31 | 7.666 | 4.327 | 3.275 | 1.835 | 4.863 | 9.471 | 7.505 |
| CODA339 | L131D | -0.902 | -7.62 | -6.009 | -3.447 | 49.328 | 47.685 | 66.7 | 64.798 | 70.85 | 69.038 |
| CODA340 | L131E | -4.061 | -0.568 | 62.964 | 63.631 | 74.912 | 75.084 | 76.346 | 75.876 | 75.805 | 74.856 |
| CODA342 | L131R | 0.271 | -3.613 | -4.935 | -4.363 | 0.282 | 2.32 | 23.777 | 18.376 | 66.17 | 66.088 |
| CODA337 | L131N | 3.093 | 12.327 | 77.097 | 77.653 | 79.446 | 79.998 | 80.691 | 80.309 | 80.258 | 79.509 |
| CODA945 | Q139E | 8.207 | 7.835 | 9.509 | 10.204 | 9.818 | 12.539 | 16.762 | 15.21 | 48.292 | 54.096 |
| CODA377 | S172D | -3.433 | -5.737 | -5.319 | -6.175 | -4.135 | -8.614 | -6.062 | -6.125 | -14.134 | -15.373 |
| CODA405 | Y210V | 7.317 | 7.643 | 9.457 | 9.134 | 15.503 | 11.954 | 13.427 | 10.784 | 9.032 | 11.591 |
| CODA407 | Y210F | 2.441 | 7.326 | 1.275 | 12.96 | 13.119 | 2.355 | 11.539 | 6.571 | 8.397 | 7.309 |
| CODA409 | Y210W | 7.776 | -2.964 | -5.568 | 3.024 | 2.815 | 3.06 | 1.766 | 2.819 | 1.844 | -4.059 |

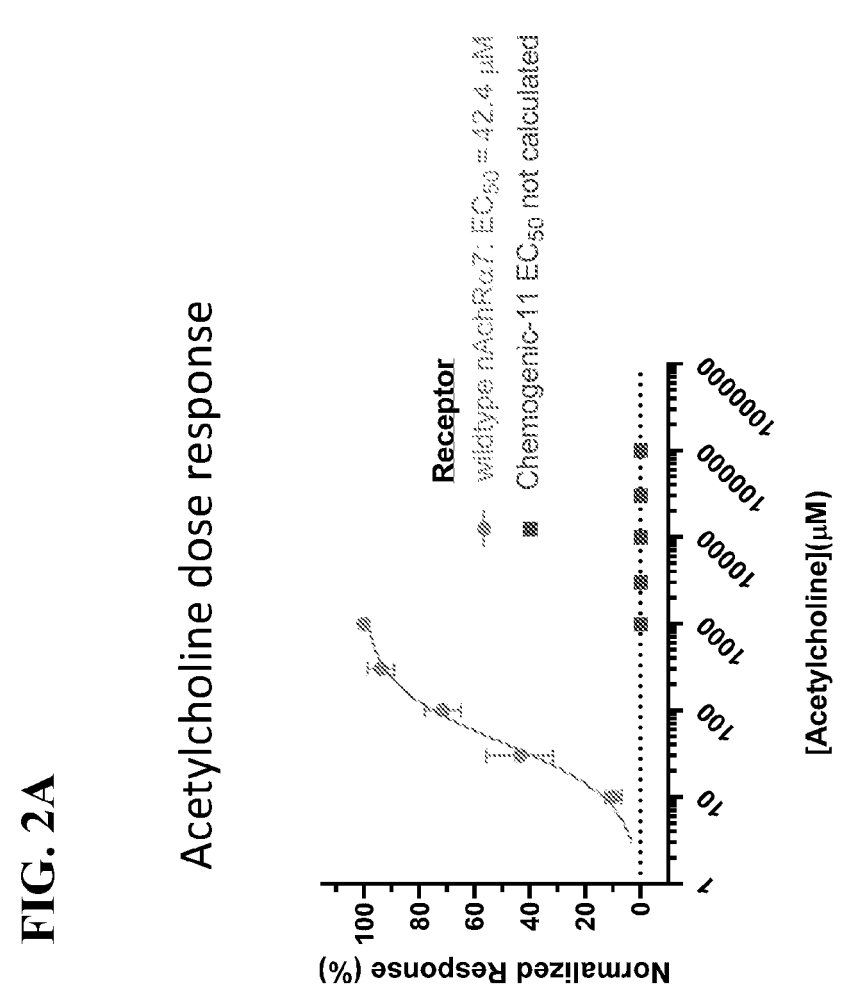
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**FIG. 1J (continued)**

| Name | Amino Acid Mutation(s) in SEQ ID NO: 33 | Varenicline | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3nM var | 3nM var | 30nM var | 30nM var | 300nM var | 300nM var | 500nM var | 500nM var | 1uM var | 1uM var |
| CODA952 | Y140I | -22.522 | -18.173 | -15.306 | -12.832 | -14.671 | -17.016 | -16.713 | -18.893 | -15.847 | -16.236 |
| CODA965 | Y140C | -17.716 | -23.019 | -14.993 | -12.182 | -11.731 | -10.13 | -12.518 | -15.043 | -13.385 | -17.022 |
| CODA1025 | R101F_L131G | 48.074 | 60.978 | 70.023 | 69.433 | 78.989 | 79.21 | 79.212 | 79.771 | 79.538 | 80.577 |
| CODA1027 | R101F_L131D | -3.367 | -0.431 | -0.761 | 2.989 | 2.966 | 10.211 | 15.278 | 20.84 | 37.104 | 42.439 |
| CODA1039 | Y115E_Y210W | -10.926 | -7.395 | -5.661 | -1.244 | -4.555 | 1.081 | 9.172 | 12.561 | 7.139 | 12.664 |
| CODA1045 | R101W_Y210V | 6.745 | 8.397 | 14.1 | 19.095 | 16.83 | 21.507 | 21.586 | 23.131 | 31.591 | 26.479 |
| CODA1047 | R101F_Y210V | -3.035 | -0.786 | -5.181 | 0.669 | -2.748 | -6.708 | -9.123 | -9.869 | -6.702 | -7.26 |
| CODA1048 | R101F_Y210F | -17.119 | -13.234 | -7.747 | 4.193 | 4.149 | 1.512 | -1.572 | 3.71 | 8.414 | 4.091 |
| CODA1053 | R101M_L131A | 74.835 | 76.119 | 77.608 | 79.015 | 80.71 | 79.002 | 78.709 | 78.884 | 79.688 | 80 |
| CODA1054 | R101M_L131F | 34.923 | 28.944 | 42.214 | 43.015 | 47.059 | 52.768 | 59.542 | 62.641 | 71.449 | 69.145 |
| CODA1055 | R101W_Y115E_Y210W | 0.468 | -10.015 | -15.532 | -14.462 | -17.027 | -15.59 | -13.343 | -10.702 | -6.604 | -7.612 |
| CODA1056 | R101F_Y115E_Y210W | | | | | | | | | | |
| CODA1138 | W77F_R101F_L131D | 3.893 | 1.107 | 0.972 | 0.872 | 2.282 | -0.288 | 3.637 | -1.804 | -1.84 | 6.36 |
| CODA1140 | R101F_L131N_S172D | -6.169 | -10.445 | -9.679 | -10.878 | -9.572 | -8.873 | -14.656 | -11.304 | -13.996 | -16.128 |
| CODA1157 | Q139E_S172D | -0.225 | -1.954 | 1.269 | 5.972 | 6.84 | 5.943 | 8.717 | 6.41 | 5.635 | 7.068 |
| CODA1173 | S172D_Y210W | 9.211 | 7.193 | 7.821 | 9.078 | 6.933 | 8.36 | 5.501 | 7.04 | 7.174 | 5.185 |

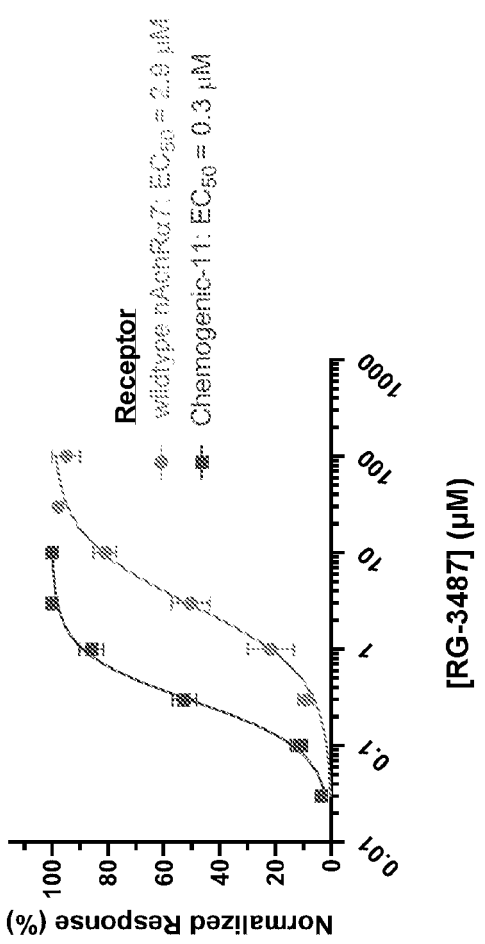
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FIG. 2A




Chemogenic-11 (CR-11):
SEQ ID NO:33 with amino acid substitutions Y115D+L131Q

FIG. 2B



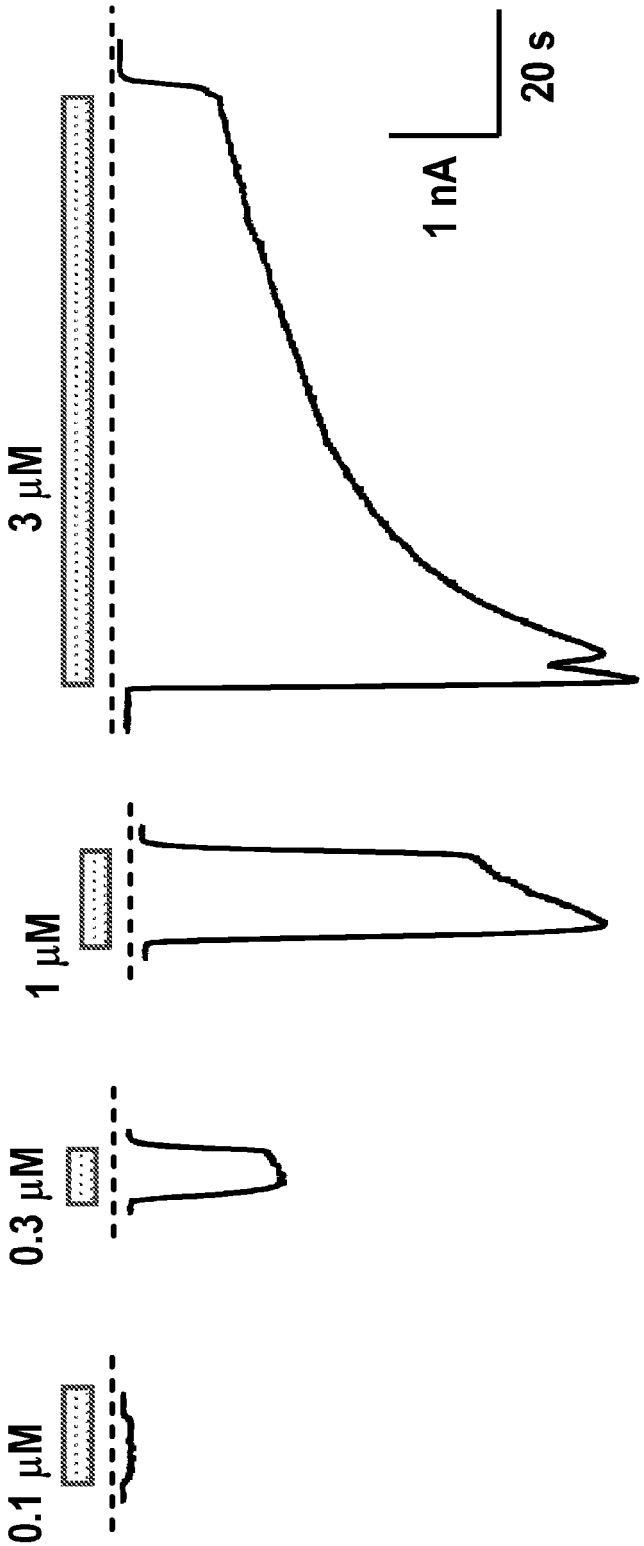



FIG. 3

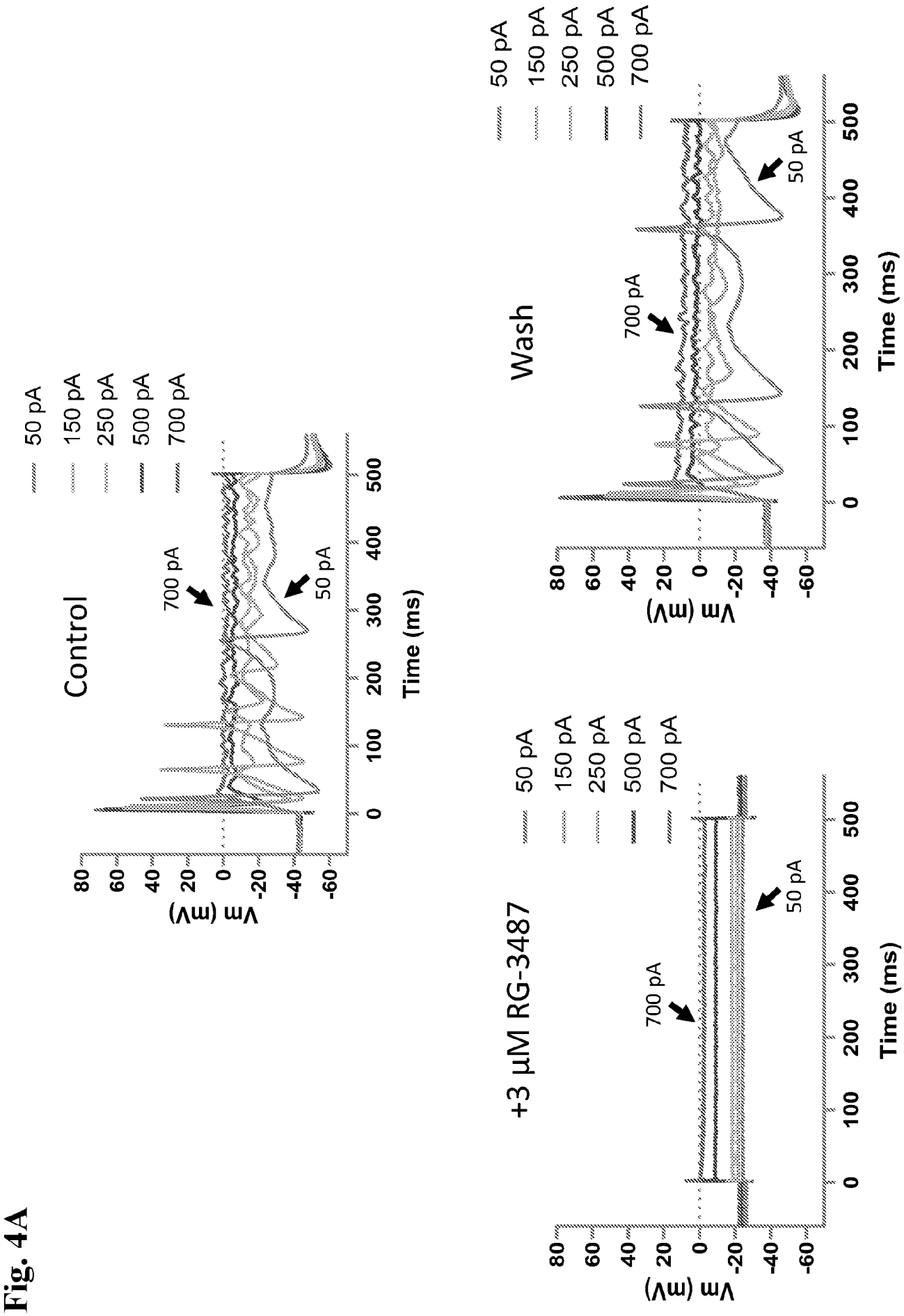

Control
+3 µM RG-3487
Wash
50 pA
150 pA
250 pA
500 pA
700 pA
700 pA
50 pA
Vm (mV)
Time (ms)
80
60
40
20
0
-20
-40
-60
0
100
200
300
400
500

Fig. 4A

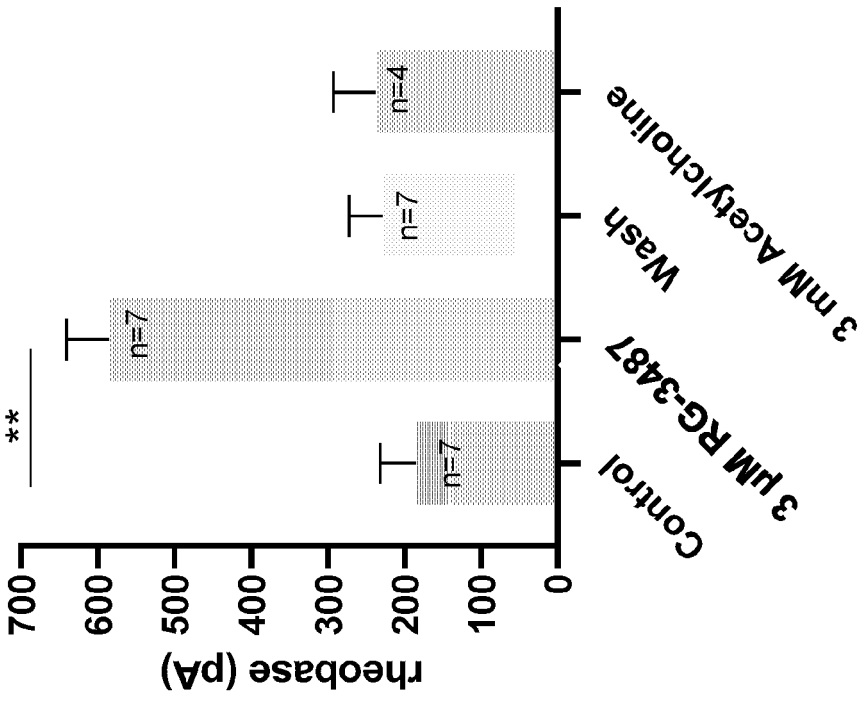

rheobase (pA)
700
600
500
400
300
200
100
0
**
n=7
n=7
n=7
n=4
Control
3 μM RG-3487
Wash
3 mM Acetylcholine

FIG. 4B

# FIG. 5

| Sequence of the Engineered Receptor | Normalized HA % | Normalized HA MFI | N | Normalized AB % | Normalized AB MFI | N |
|---|---|---|---|---|---|---|
| Y115D in SEQ ID NO: 33 | 0.91 | 0.74 | 2 | 1.23 | 1.04 | 2 |
| Y115E in SEQ ID NO: 33 | 1.16 | 1.00 | 2 | 1.24 | 1.31 | 2 |
| L131S in SEQ ID NO: 33 | 1.42 | 0.76 | 2 | 1.28 | 1.20 | 2 |
| L131T in SEQ ID NO: 33 | 2.00 | 0.88 | 2 | 1.65 | 1.32 | 3 |
| L131D in SEQ ID NO: 33 | 1.59 | 0.79 | 2 | 0.84 | 1.41 | 3 |
| L131R in SEQ ID NO: 33 | 1.83 | 0.74 | 2 | 0.61 | 1.24 | 3 |
| S172D in SEQ ID NO: 33 | 0.36 | 0.61 | 2 | 0.50 | 0.90 | 4 |
| Y210W in SEQ ID NO: 33 | 0.96 | 1.09 | 2 | 0.88 | 0.62 | 4 |
| L131S_S172D in SEQ ID NO: 33 | 0.47 | 1.10 | 8 | 0.44 | 0.91 | 3 |
| L131T_S172D in SEQ ID NO: 33 | 0.33 | 0.86 | 9 | 0.43 | 0.72 | 2 |
| L131D_S172D in SEQ ID NO: 33 | 0.40 | 0.87 | 9 | 0.48 | 0.87 | 2 |
| Y115D_S170T in SEQ ID NO: 33 | 0.70 | 0.94 | 4 | 0.56 | 0.93 | 4 |
| Y115D_L131Q in SEQ ID NO: 33 | 0.36 | 0.83 | 4 | 0.33 | 2.04 | 5 |
| Y115D_L131E in SEQ ID NO: 33 | 0.91 | 1.00 | 4 | 0.78 | 1.14 | 4 |

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# FIG. 5 (continued)

| Sequence of the Engineered Receptor | Normalized HA % | Normalized HA MFI | N | Normalized AB % | Normalized AB MFI | N |
|---|---|---|---|---|---|---|
| Y140I in SEQ ID NO: 33 | 1.41 | 1.53 | 2 | 0.54 | 2.64 | 2 |
| R101F_L131G in SEQ ID NO: 33 | 0.98 | 0.63 | 2 | 0.46 | 0.90 | 2 |
| R101F_L131D in SEQ ID NO: 33 | 0.61 | 0.49 | 2 | 0.50 | 0.68 | 2 |
| Y115E_Y210W in SEQ ID NO: 33 | 1.44 | 0.68 | 2 | 0.34 | 1.08 | 2 |
| R101W_Y210V in SEQ ID NO: 33 | 1.09 | 0.87 | 11 | 0.03 | 0.35 | 12 |
| R101F_Y210V in SEQ ID NO: 33 | 1.13 | 0.65 | 4 | 0.03 | 0.38 | 4 |
| R101F_Y210F in SEQ ID NO: 33 | 1.05 | 0.61 | 4 | 0.55 | 0.57 | 4 |
| R101M_L131A in SEQ ID NO: 33 | 1.31 | 0.97 | 10 | 0.91 | 0.90 | 10 |
| R101M_L131F in SEQ ID NO: 33 | 0.96 | 0.88 | 11 | 0.82 | 0.82 | 12 |
| R101W_Y115E_Y210W in SEQ ID NO: 33 | 1.07 | 0.78 | 9 | 0.51 | 0.69 | 9 |
| R101F_Y115E_Y210W in SEQ ID NO: 33 | 1.22 | 0.56 | 4 | 0.44 | 0.70 | 4 |

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# FIG. 6A

| Engineered Receptor | Relative Total Expression in Cultured DRG neurons* (normalized to wildtype glycine receptor) | | Relative Surface Expression in Cultured DRG neurons* (normalized to wildtype glycine receptor) | | N coverslips | Expression ratio in Cultured DRG neurons* | | Relative Surface Expression in HEK Cells (normalized to SEQ ID NO: 33) | |
|---|---|---|---|---|---|---|---|---|---|
| | Soma | Processes | Soma | Processes | | Total HA, axon : soma | Soma surface : total | % HA+ | HA MFI |
| **lentiviral vectors** | | | | | | | | | |
| Wildtype Glycine Receptor a1 | 1 | 1 | 1 | 1 | 7 | | | | |
| L131R in SEQ ID NO: 33 | 0.33 ± .02 | 0.18 ± .03 | 0.55 ± .18 | 0.89 ± .50 | 5 | | | 2.28 ± .1.65 | 0.80 ± 0.20 |
| Y210W in SEQ ID NO: 33 | 0.28 ± .03 | 0.12 ± .01 | 0.35 ± .06 | 0.30 ± .06 | 7 | | | 0.80 ± .0.23 | 0.76 ± .0.11 |
| Y115E in SEQ ID NO: 33 | 0.45 ± .07 | (0) | 0.29 ± .06 | 0.25 ± .02 | 4 | | | 1.03 ± .16 | 1.07 ± .0.05 |
| L131D in SEQ ID NO: 33 | 0.37 ± .07 | 0.19 ± .06 | 0.22 ± .05 | 0.35 ± .11 | 6 | | | 2.06 ± 1.55 | 1.04 ± .12 |
| R101F, L131G in SEQ ID NO: 33 | 0.51 ± .09 | (0) | 0.25 ± .05 | 0.08 ± .05 | 4 | | | 0.78 ± .0.05 | 0.89 ± .0.01 |
| R101F, L131D in SEQ ID NO: 33 | 0.19 ± .04 | (0) | 0.20 ± .05 | 0.08 ± .07 | 6 | | | 0.48 ± .0.01 | 0.69 ± .0.04 |
| Y115E, Y210W in SEQ ID NO: 33 | 0.33 ± .03 | 0.09 ± .02 | 0.36 ± .05 | 0.51 ± .04 | 4 | | | | |
| empty vector | 0.09 ± .03 | 0.01 ± .03 | 0.30 ± .10 | 0.15 ± .07 | 7 | | | | |
| | | | | | | | | | |
| **AAV6 vectors** | | | | | | | | | |
| Wildtype Glycine Receptor a1 | 1 | 1 | 1 | 1 | 12 | 0.07 ± .01 | 1.01 ± .06 | | |
| SEQ ID NO: 33 | 0.44 ± .10 | 0.18 ± .08 | 0.26 ± .07 | 0.26 ± .21 | 5 | 0.01 ± .00 | 0.55 ± .12 | | |
| Y210W in SEQ ID NO: 33 | 0.35 ± .04 | 0.16 ± .02 | 0.14 ± .06 | 0.10 ± .08 | 4 | 0.03 ± .00 | 0.43 ± .18 | 0.80 ± .0.23 | 0.76 ± .0.11 |
| R101W, Y210V in SEQ ID NO: 33 | 0.62 ± .06 | 0.33 ± .08 | 0.40 ± .12 | 0.52 ± .14 | 5 | 0.02 ± .00 | 0.73 ± .24 | 0.99 ± .0.36 | 0.99 ± .0.14 |
| R101F, Y210F in SEQ ID NO: 33 | 0.46 ± .17 | 0.05 ± .02 | 0.06 ± .06 | 0.01 ± .1 | 3 | 0.01 ± .00 | 0.24 ± .25 | 0.90 ± .0.32 | 0.95 ± .0.15 |
| R101M, L131F in SEQ ID NO: 33 | 0.31 ± .05 | 0.17 ± .04 | 0.24 ± .03 | 0.39 ± .14 | 6 | 0.02 ± .00 | 0.78 ± .06 | 0.92 ± .0.38 | 1.06 ± .0.15 |
| R101W,Y115E,Y210W in SEQ ID NO: 33 | 0.39 ± .04 | 0.22 ± .03 | 0.30 ± .04 | 0.39 ± .14 | 6 | 0.03 ± .00 | 0.77 ± .06 | 0.78 ± .0.10 | 0.84 ± 0.10 |
| R101F,Y115E,Y210W in SEQ ID NO: 33 | 0.49 ± .02 | 0.11 ± .04 | 0.31 ± .06 | 0.08 ± .08 | 6 | 0.02 ± .01 | 0.63 ± .11 | | |
| L131G, Q139L, Y217F in SEQ ID NO: 16 | 0.28 ± .06 | 0.01 ± .01 | 0.04 ± .03 | 0.02 ± .03 | 4 | 0.00 ± .00 | 0.10 ± .08 | 1.02 ± .23 | 1.01 ± .11 |





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# FIG. 6B

| Engineered Receptor | Relative Total Expression in Cultured hippocampal neurons* (normalized to wildtype glycine receptor) | | Relative Surface Expression in Cultured hippocampal neurons* (normalized to wildtype glycine receptor) | | N coverslips |
|---|---|---|---|---|---|
| | Soma | Processes | Soma | Processes | |
| **lentiviral vectors** | | | | | |
| Wildtype Glycine Receptor a1 | 1 | 1 | 1 | 1 | 12 |
| SEQ ID NO: 33 | 0.44 ± .12 | 0.30 ± .08 | 0.61 ± .22 | 0.36 ± .11 | 4 |
| L131R in SEQ ID NO: 33 | 0.65 ± .13 | 0.34 ± .07 | 0.29 ± .06 | 0.17 ± .03 | 5 |
| Y210W in SEQ ID NO: 33 | 0.60 ± .05 | 0.27 ± .02 | 0.29 ± .05 | 0.18 ± .04 | 6 |
| Y115E in SEQ ID NO: 33 | 0.58 ± .11 | 0.30 ± .04 | 0.42 ± .02 | 0.32 ± .03 | 4 |
| L131D in SEQ ID NO: 33 | 0.54 ± .13 | 0.31 ± .06 | 0.29 ± .03 | 0.21 ± .03 | 6 |
| R101F, L131G in SEQ ID NO: 33 | 0.38 ± .06 | 0.18 ± .02 | 0.38 ± .05 | 0.23 ± .02 | 3 |
| R101F, L131D in SEQ ID NO: 33 | 0.24 ± 08 | 0.05 ± 03 | 0.10 ± .02 | 0.06 ± .02 | 2 |
| Y115E, Y210W in SEQ ID NO: 33 | 0.29 | 0.19 | 0.48 | 0.35 | 1 |
| | | | | | |
| **AAV6 vectors** | | | | | |
| Wildtype Glycine Receptor a1 | 1 | 1 | 1 | 1 | 8 |
| SEQ ID NO: 33 | 0.67 ± .26 | 0.35 ± .11 | 0.34 ± .11 | 0.23 ± .06 | 4 |
| Y210W in SEQ ID NO: 33 | 0.65 ± .12 | 0.27 ± .03 | 0.24 ± .04 | 0.11 ± .02 | 4 |
| R101M, L131F in SEQ ID NO: 33 | 0.57 ± .07 | 0.26 ± .02 | 0.12 ± .03 | 0.15 ± .11 | 4 |
| R101W,Y115E,Y210W in SEQ ID NO: 33 | 0.39 ± .08 | 0.14 ± .02 | 0.13 ± .05 | 0.06 ± .03 | 4 |
| R101F,Y115E,Y210W in SEQ ID NO: 33 | 0.38 ± .04 | 0.38 ± .12 | 0.13 ± .00 | 0.13 ± .13 | 2 |
| L131G, Q139L, Y217F in SEQ ID NO: 16 | 0.38 ± .12 | (-0.04 ± .04) | (-0.11 ± .07) | (-0.11 ± .04) | 4 |

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# Sequence Listing

| | | |
|---|---|---|
| **1** | **Sequence Listing Information** | |
| 1-1 | File Name | SWCH_039_01WO_SeqList_ST26.xml |
| 1-2 | DTD Version | V1_3 |
| 1-3 | Software Name | WIPO Sequence |
| 1-4 | Software Version | 2.2.0 |
| 1-5 | Production Date | 2023-02-17 |
| 1-6 | Original free text language code | en |
| 1-7 | Non English free text language code | |
| **2** | **General Information** | |
| 2-1 | Current application: IP Office | WO |
| 2-2 | Current application: Application number | |
| 2-3 | Current application: Filing date | |
| 2-4 | Current application: Applicant file reference | SWCH-039/01WO 322917-2493 |
| 2-5 | Earliest priority application: IP Office | US |
| 2-6 | Earliest priority application: Application number | 63/311,817 |
| 2-7 | Earliest priority application: Filing date | 2022-02-18 |
| 2-8en | Applicant name | Trames Bio, Inc. |
| 2-8 | Applicant name: Name Latin | |
| 2-9en | Inventor name | |
| 2-9 | Inventor name: Name Latin | |
| 2-10en | Invention title | COMPOSITIONS AND METHODS FOR NEUROLOGICAL DISEASES |
| 2-11 | Sequence Total Quantity | 98 |

| | | |
|---|---|---|
| **3-1** | **Sequences** | |
| 3-1-1 | Sequence Number [ID] | 1 |
| 3-1-2 | Molecule Type | DNA |
| 3-1-3 | Length | 1835 |
| 3-1-4 | Features Location/ | **source 1..1835** |
| | Qualifiers | mol_type=other DNA |
| | | organism=Homo sapiens |
| | NonEnglishQualifier Value | |
| 3-1-5 | Residues | caacagacac gctggagttt aacaaacagc aatactcttc gcgctcctga aaagcaggtc 60<br>tggacgctct ccgtggtgct gaaacgcctc gcagccgccg ctgtccgtgg tatctacgac 120<br>cccctcgctc caatttcccc tggggctctc cctccgcgcc cctgttcccc gcctcccttt 180<br>aacatctgga ttatttttg caatagcgct ttctggtttt gtaagtgcca atttgaaaca 240<br>tttttgcccc cataactcgt ggactacaaa gcacaaggac ctgaaaaatg tacagcttca 300<br>atactcttcg actctacctt tgggagacca ttgtattctt cagccttgct gcttctaagg 360<br>aggctgaagc tgctcgctcc gcacccaagc ctatgtcacc ctcggatttc ctggataagc 420<br>taatggggag aacctccgga tatgatgcca ggatcaggcc caattttaaa ggtcccccag 480<br>tgaacgtgag ctgcaacatt ttcatcaaca gctttggttc cattgctgag acaaccatgg 540<br>actatagggt caacatcttc ctgcggcagc aatggaacga cccccgcctg gcctataatg 600<br>aataccctga cgactctctg gacctggacc catccatgct ggactccatc tggaaacctg 660<br>acctgttctt tgccaacgag aaggggccc acttccatga gatcaccaca gacaacaaat 720<br>tgctaaggat ctcccggaat gggaatgtcc tctacagcat cagaatcacc ctgacactgg 780<br>cctgccccat ggacttgaag aatttcccca tggatgtcca gacatgtatc atgcaactgg 840<br>aaagctttgg atatacgatg aatgacctca tctttgagtg gcaggaacag ggagccgtgc 900<br>aggtagcaga tggactaact ctgccccagt ttatcttgaa ggaagagaag gacttgagat 960<br>actgcaccaa gcactacaac acaggtaaat tcacctgcat tgaggcccgg ttccacctgg 1020<br>agcggcagat gggttactac ctgattcaga tgtatattcc cagcctgctc attgtcatcc 1080<br>tctcatggat ctccttctgg atcaacatgg atgctgcacc tgctcgtgtg ggcctaggca 1140<br>tcaccactgt gctcaccatg accacccaga gctccggctc tcgagcatct ctgcccaagg 1200<br>tgtcctatgt gaaagccatt gacatttgga tggcagtttg cctgctcttt gtgttctcag 1260<br>ccctattaga atatgctgcc gttaactttg tgtctcggca acataaggag ctgctccgat 1320<br>tcaggaggaa gcggagacat cacaagagcc ccatgttgaa tctattccag gaggatgaag 1380<br>ctggagaagg ccgctttaac ttctctgcct atgggatggg cccagcctgt ctacaggcca 1440<br>aggatggcat ctcagtcaag ggcgccaaca acagtaacac caccaacccc cctcctgcac 1500<br>catctaagtc cccagaggag atgcgaaaac tcttcatcca gagggccaag aagatcgaca 1560<br>aaatatcccg cattggcttc cccatggcct tcctcatttt caacatgttc tactggatca 1620<br>tctacaagat tgtccgtaga gaggacgtcc acaaccagtg aagggtctga aaggttgggg 1680<br>gaggctggga gaggggaacg tgggaatagc acaggaatct gagagactaa ggaagagaag 1740<br>gggaacggag ggaggggca cacttacaca actctctctg caatatgtgc aatagcaaaa 1800<br>tgcagtgatg catgaatttt aaaaaaaaaa aaaaa 1835 |
| **3-2** | **Sequences** | |
| 3-2-1 | Sequence Number [ID] | 2 |
| 3-2-2 | Molecule Type | AA |
| 3-2-3 | Length | 457 |
| 3-2-4 | Features Location/ | **source 1..457** |
| | Qualifiers | mol_type=protein |
| | | organism=Homo sapiens |
| | NonEnglishQualifier Value | |
| 3-2-5 | Residues | MYSFNTLRLY LWETIVFFSL AASKEAEAAR SAPKPMSPSD FLDKLMGRTS GYDARIRPNF 60<br>KGPPVNVSCN IFINSFGSIA ETTMDYRVNI FLRQQWNDPR LAYNEYPDDS LDLDPSMLDS 120<br>IWKPDLFFAN EKGAHFHEIT TDNKLLRISR NGNVLYSIRI TLTLACPMDL KNFPMDVQTC 180<br>IMQLESFGYT MNDLIFEWQE QGAVQVADGL TLPQFILKEE KDLRYCTKHY NTGKFTCIEA 240<br>RFHLERQMGY YLIQMYIPSL LIVILSWISF WINMDAAPAR VGLGITTVLT MTTQSSGSRA 300<br>SLPKVSYVKA IDIWMAVCLL FVFSALLEYA AVNFVSRQHK ELLRFRRKRR HHKSPMLNLF 360<br>QEDEAGEGRF NFSAYGMGPA CLQAKDGISV KGANNSNTTN PPPAPSKSPE EMRKLFIQRA 420<br>KKIDKISRIG FPMAFLIFNM FYWIIYKIVR REDVHNQ 457 |
| **3-3** | **Sequences** | |
| 3-3-1 | Sequence Number [ID] | 3 |
| 3-3-2 | Molecule Type | DNA |
| 3-3-3 | Length | 3356 |
| 3-3-4 | Features Location/ | **source 1..3356** |
| | Qualifiers | mol_type=other DNA |
| | | organism=Homo sapiens |
| | NonEnglishQualifier Value | |
| 3-3-5 | Residues | tccttaaagg cgcgcgagcc gagcggcgag gtgcctctgt ggccgcaggc gcaggcccgg 60<br>gcgacagccg agacgtggag cgcgccggct cgctgcagct ccgggactca acatgcgctg 120 |

| | | |
|---|---|---|
| | | ctcgccggga ggcgtctggc tggcgctggc cgcgtcgctc ctgcacgtgt ccctgcaagg 180<br>cgagttccag aggaagcttt acaaggagct ggtcaagaac tacaatccct tggagaggcc 240<br>cgtggccaat gactcgcaac cactcaccgt ctacttctcc ctgagcctcc tgcagatcat 300<br>ggacgtggat gagaagaacc aagttttaac caccaacatt tggctgcaaa tgtcttggac 360<br>agatcactat ttacagtgga atgtgtcaga atatccaggg gtgaagactg ttcgtttccc 420<br>agatggccag atttggaaac cagacattct tctctataac agtgctgatg agcgctttga 480<br>cgccacattc cacactaacg tgttggtgaa ttcttctggg cattgccagt acctgcctcc 540<br>aggcatattc aagagttcct gctacatcga tgtacgctgg tttccctttg atgtgcagca 600<br>ctgcaaactg aagtttgggt cctggtctta cggaggctgg tccttggatc tgcagatgca 660<br>ggaggcagat atcagtggct atatccccaa tggagaatgg gacctagtgg gaatccccgg 720<br>caagaggagt gaaaggttct atgagtgctg caaagagccc taccccgatg tcaccttcac 780<br>agtgaccatg cgccgcagga cgctctacta tggcctcaac ctgctgatcc cctgtgtgct 840<br>catctccgcc ctcgccctgc tggtgttcct gcttcctgca gattccgggg agaagatttc 900<br>cctggggata acagtcttac tctctcttac cgtcttcatg ctgctcgtgg ctgagatcat 960<br>gcccgcaaca tccgattcgg taccattgat agcccagtac ttcgccagca ccatgatcat 1020<br>cgtgggcctc tcggtggtgg tgacagtgat cgtgctgcag taccaccacc acgaccccga 1080<br>cgggggcaag atgcccaagt ggaccagagt catccttctg aactggtgcg cgtggttcct 1140<br>gcgaatgaag aggcccgggg aggacaaggt gcgcccggcc tgccagcaca agcagcggcg 1200<br>ctgcagcctg gccagtgtgg agatgagcgc cgtggcgccg ccgcccgcca gcaacgggaa 1260<br>cctgctgtac atcggcttcc gcggcctgga cggcgtgcac tgtgtcccga cccccgactc 1320<br>tggggtagtg tgtggccgca tggcctgctc ccccacgcac gatgagcacc tcctgcacgg 1380<br>cgggcaaccc cccgaggggg acccggactt ggccaagatc ctggaggagg tccgctacat 1440<br>tgccaaccgc ttccgctgcc aggacgaaag cgaggcggtc tgcagcgagt ggaagttcgc 1500<br>cgcctgtgtg gtggaccgcc tgtgcctcat ggccttctcg gtcttcacca tcatctgcac 1560<br>catcggcatc ctgatgtcgg ctcccaactt cgtggaggcc gtgtccaaag actttgcgta 1620<br>accacgcctg gttctgtaca tgtggaaaac tcacagatgg gcaaggcctt tggcttggcg 1680<br>agatttgggg gtgctaatcc aggacagcat tacacgccac aactccagtg ttcccttctg 1740<br>gctgtcagtc gtgttgctta cggtttcttt gttactttag gtagtagaat ctcagcactt 1800<br>tgtttcatat tctcagatgg gctgatagat atccttggca catccgtacc atcggtcagc 1860<br>agggccactg agtagtcatt ttgcccatta gcccactgcc tggaaagccc ttcggagagc 1920<br>tccccatggc tcctcaccac cgagacagtt ggttttgcat gtctgcatga aggtctacct 1980<br>gaaaattcaa catttgcttt ttgcttgtgt acaaacccag attgaagcta aaataaacca 2040<br>gactcactaa atcctttcca ataattgact ggtggaagga aaacaaaaaa caaaaactaa 2100<br>aaacctctta gcttttctgc aattcaactt tttatttta tttttatttc tatcaaagac 2160<br>ggtagagaga aacagcttga tgctgtttct acattaaaaa aaaaaaaaaa agacagactg 2220<br>ttggtcttac taaggatgtt tttaccagcc tgcctgactt ctgcaaacct accctgtcaa 2280<br>ggagatcaaa gggacgcagg tttctgttta ttctgaacaa gggccaggcc ccgcggagtg 2340<br>tctttggtgg atcccagata actcctaggt gctgctctca gacactgagg agttgagcaa 2400<br>atctgttcta ttctgcagaa cccacaggac aaataagagt tctactagaa ttaacagccc 2460<br>aaaagaatag ctacagctaa gtgaagccac ttacgtgggc tttaaaaaaa taatgtgtta 2520<br>gctgattcac atgcactgga gttaattagt cttagaaatg tgtgcatcca tacaaatgca 2580<br>caacataaag tgaacatatt cctaggccct ttctgcctgt gtcagggcca ggaagtagag 2640<br>gctgggaact cttctggtcc ccagtatggc aggcgccagg gaggggatgg tgtggcccat 2700<br>cccttctctg gatacctggc cagtggcagg cagcagggag gagctggccg accctcagtg 2760<br>actgacaagc cagcaattct gagttctggc ctttgggagt ctgcctgctc caagccagtc 2820<br>caccccagct gcagccccaa aagctggctc aaagtccttg ggtggattca ctggagatgg 2880<br>gcaacttaaa acaagagaaa ctttaatttt taaacctaag tgatgataca gctcttccct 2940<br>tagattatcg cccaggctgg agtgcagtgg catgatctca gctcactgca agctccacct 3000<br>cctgggttca tgccattctc ctgcctcagc ctccccccga gtaactggga atacaggcgc 3060<br>ccgccaccat gcctggctaa ttttttgtat ttttagtaga gatagggttt catcatgtta 3120<br>gccaggatgg tctcattctt attctttaat gagatcagag ggtaattcac caagaaagac 3180<br>ctctcctgtt ccattgtgtc atccaacaac tgctcagagc tcaaaattat agaaggcttc 3240<br>tgagccccta gagattttta atttgcttct aatccctgag gtgggaacat catgagggaa 3300<br>gatttgattt tcagagttaa ataaattgta tgtgcttttc cagccaaaaa aaaaaa 3356 |
| **3-4** | **Sequences** | |
| 3-4-1 | Sequence Number [ID] | 4 |
| 3-4-2 | Molecule Type | AA |
| 3-4-3 | Length | 502 |
| 3-4-4 | Features Location/ | **source 1..502** |
| | Qualifiers | mol_type=protein |
| | | organism=Homo sapiens |
| | NonEnglishQualifier Value | |
| 3-4-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDEKNQ VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV RFPDGQIWKP DILLYNSADE 120<br>RFDATFHTNV LVNSSGHCQY LPPGIFKSSC YIDVRWFPFD VQHCKLKFGS WSYGGWSLDL 180 |

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|---|---|---|
| | | QMQEADISGY IPNGEWDLVG IPGKRSERFY ECCKEPYPDV TFTVTMRRRT LYYGLNLLIP 240<br>CVLISALALL VFLLPADSGE KISLGITVLL SLTVFMLLVA EIMPATSDSV PLIAQYFAST 300<br>MIIVGLSVVV TVIVLQYHHH DPDGGKMPKW TRVILLNWCA WFLRMKRPGE DKVRPACQHK 360<br>QRRCSLASVE MSAVAPPPAS NGNLLYIGFR GLDGVHCVPT PDSGVVCGRM ACSPTHDEHL 420<br>LHGGQPPEGD PDLAKILEEV RYIANRFRCQ DESEAVCSEW KFAACVVDRL CLMAFSVFTI 480<br>ICTIGILMSA PNFVEAVSKD FA 502 |
| **3-5** | **Sequences** | |
| 3-5-1 | Sequence Number [ID] | 5 |
| 3-5-2 | Molecule Type | DNA |
| 3-5-3 | Length | 2343 |
| 3-5-4 | Features Location/ Qualifiers | **source 1..2343**<br>mol_type=other DNA<br>organism=Homo sapiens |
| | NonEnglishQualifier Value | |
| 3-5-5 | Residues | ctctcagctg tcccctcccc tttcctcccg cctgaaacat gatccagctg aaggactgat 60<br>tgcaggaaaa cttggcagct ccccaacctt ggtggcccag ggagtgtgag gctgcagcct 120<br>cagaaggtgt gagcagtggc cacgagaggc aggctggctg ggacatgagg ttggcagagg 180<br>gcaggcaagc tggcccttgg tgggcctcgt cctgagcact cggaggcact cctatgcttg 240<br>gaaagctcgc tatgctgctg tgggtccagc aggcgctgct cgccttgctc ctccccacac 300<br>tcctggcaca gggagaagcc aggaggagcc gaaacaccac caggcccgct ctgctgaggc 360<br>tgtcggatta ccttttgacc aactacagga agggtgtgcg ccccgtgagg gactggagga 420<br>agccaaccac cgtatccatt gacgtcattg tctatgccat cctcaacgtg gatgagaaga 480<br>atcaggtgct gaccacctac atctggtacc ggcagtactg gactgatgag tttctccagt 540<br>ggaaccctga ggactttgac aacatcacca agttgtccat ccccacggac agcatctggg 600<br>tcccggacat tctcatcaat gagttcgtgg atgtggggaa gtctccaaat atcccgtacg 660<br>tgtatattcg gcatcaaggc gaagttcaga actacaagcc ccttcaggtg gtgactgcct 720<br>gtagcctcga catctacaac ttccccttcg atgtccagaa ctgctcgctg accttcacca 780<br>gttggctgca caccatccag gacatcaaca tctctttgtg gcgcttgcca gaaaaggtga 840<br>aatccgacag gagtgtcttc atgaaccagg gagagtggga gttgctgggg gtgctgccct 900<br>actttcggga gttcagcatg gaaagcagta actactatgc agaaatgaag ttctatgtgg 960<br>tcatccgccg gcggcccctc ttctatgtgg tcagcctgct actgcccagc atcttcctca 1020<br>tggtcatgga catcgtgggc ttctacctgc cccccaacag tggcgagagg gtctctttca 1080<br>agattacact cctcctgggc tactcggtct tcctgatcat cgtttctgac acgctgccgg 1140<br>ccactgccat cggcactcct ctcattggta aggcccctcc tggcagcaga gctcagtctg 1200<br>gtgagaaacc cgccccctcc cacctcctgc atgtgtctct tgcctctgcc ctgggctgca 1260<br>caggtgtcta ctttgtggtg tgcatggctc tgctggtgat aagtttggcc gagaccatct 1320<br>tcattgtgcg gctggtgcac aagcaagacc tgcagcagcc cgtgcctgct tggctgcgtc 1380<br>acctggttct ggagagaatc gcctggctac tttgcctgag ggagcagtca acttcccaga 1440<br>ggcccccagc cacctcccaa gccaccaaga ctgatgactg ctcagccatg ggaaaccact 1500<br>gcagccacat gggaggaccc caggacttcg agaagagccc gagggacaga tgtagccctc 1560<br>ccccaccacc tcgggaggcc tcgctggcgg tgtgtgggct gctgcaggag ctgtcctcca 1620<br>tccggcaatt cctggaaaag cgggatgaga tccgagaggt ggcccgagac tggctgcgcg 1680<br>tgggctccgt gctggacaag ctgctattcc acatttacct gctagcggtg ctggcctaca 1740<br>gcatcaccct ggttatgctc tggtccatct ggcagtacgc ttgagtgggt acagcccagt 1800<br>ggaggagggg gtacagtcct ggttaggtgg ggacagagga tttctgctta ggcccctcag 1860<br>gacccaggga atgccaggga cattttcaag acacagacaa agtcccgtgc cctgtttcca 1920<br>atgccaattc atctcagcaa tcacaagcca aggtctgaac ccttccacca aaaactgggt 1980<br>gttcaaggcc cttacaccct tgtcccaccc ccagcagctc accatggctt taaaacatgc 2040<br>tgtcttagat caggagaaac tcgggcactc cctaagtcca ctctagttgt ggacttttcc 2100<br>ccattgaccc tcacctgaat aagggacttt ggaattctgc ttctctttca caactttgct 2160<br>tttaggttga aggcaaaacc aactctctac tacacaggcc tgataactct gtacgaggct 2220<br>tctctaaccc ctagtgtctt ttttttcttc acctcacttg tggcagcttc cctgaacact 2280<br>catcccccat cagatgatgg gagtgggaag aataaaatgc agtgaaaccc taaaaaaaaa 2340<br>aaa 2343 |
| **3-6** | **Sequences** | |
| 3-6-1 | Sequence Number [ID] | 6 |
| 3-6-2 | Molecule Type | AA |
| 3-6-3 | Length | 516 |
| 3-6-4 | Features Location/ Qualifiers | **source 1..516**<br>mol_type=protein<br>organism=Homo sapiens |
| | NonEnglishQualifier Value | |
| 3-6-5 | Residues | MLGKLAMLLW VQQALLALLL PTLLAQGEAR RSRNTTRPAL LRLSDYLLTN YRKGVRPVRD 60<br>WRKPTTVSID VIVYAILNVD EKNQVLTTYI WYRQYWTDEF LQWNPEDFDN ITKLSIPTDS 120<br>IWVPDILINE FVDVGKSPNI PYVYIRHQGE VQNYKPLQVV TACSLDIYNF PFDVQNCSLT 180 |

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| | | FTSWLHTIQD INISLWRLPE KVKSDRSVFM NQGEWELLGV LPYFREFSME SSNYYAEMKF 240<br>YVVIRRRPLF YVVSLLLPSI FLMVMDIVGF YLPPNSGERV SFKITLLLGY SVFLIIVSDT 300<br>LPATAIGTPL IGKAPPGSRA QSGEKPAPSH LLHVSLASAL GCTGVYFVVC MALLVISLAE 360<br>TIFIVRLVHK QDLQQPVPAW LRHLVLERIA WLLCLREQST SQRPPATSQA TKTDDCSAMG 420<br>NHCSHMGGPQ DFEKSPRDRC SPPPPPREAS LAVCGLLQEL SSIRQFLEKR DEIREVARDW 480<br>LRVGSVLDKL LFHIYLLAVL AYSITLVMLW SIWQYA 516 |
| **3-7** | **Sequences** | |
| 3-7-1 | Sequence Number [ID] | 7 |
| 3-7-2 | Molecule Type | DNA |
| 3-7-3 | Length | 5723 |
| 3-7-4 | Features Location/ | **source 1..5723** |
| | Qualifiers | mol_type=other DNA |
| | | organism=Homo sapiens |
| | NonEnglishQualifier Value | |
| 3-7-5 | Residues | gctccgggcc agcgcggcgg cggcggcggc ggcggcagca gcaggagcag ccccggctgc 60<br>gggtcgcgac ggcggcgggg cgccccctcc cccgtgccgg ggcgcggcgg agggatgtgg 120<br>ggccttgcgg gaggaaggct tttcggcatc ttctcggccc cggtgctggt ggctgtggtg 180<br>tgctgcgccc agagtgtgaa cgatcccggg aacatgtcct ttgtgaagga gacggtggac 240<br>aagctgttga aaggctacga cattcgccta agacccgact tcgggggtcc cccggtctgc 300<br>gtggggatga acatcgacat cgccagcatc gacatggttt ccgaagtcaa catggattat 360<br>accttaacca tgtattttca acaatattgg agagataaaa ggctcgccta ttctgggatc 420<br>cctctcaacc tcacgcttga caatcgagtg gctgaccagc tatgggtgcc cgacacatat 480<br>ttcttaaatg acaaaaagtc atttgtgcat ggagtgacag tgaaaaaccg catgatccgt 540<br>cttcaccctg atgggacagt gctgtatggg ctcagaatca ccacgacagc agcatgcatg 600<br>atggacctca ggagataccc cctggacgag cagaactgca ctctggaaat tgaaagctat 660<br>ggctacacca cggatgacat tgagttttac tggcgaggcg gggacaaggc tgttaccgga 720<br>gtggaaagga ttgagctccc gcagttctcc atcgtggagc accgtctggt ctcgaggaat 780<br>gttgtcttcg ccacaggtgc ctatcctcga ctgtcactga gctttcggtt gaagaggaac 840<br>attggatact tcattcttca gacttatatg ccctctatac tgataacgat tctgtcgtgg 900<br>gtgtccttct ggatcaatta tgatgcatct gctgctagag ttgccctcgg gatcacaact 960<br>gtgctgacaa tgacaaccat caacacccac cttcgggaga ccttgcccaa aatcccctat 1020<br>gtcaaagcca ttgacatgta ccttatgggc tgcttcgtct ttgtgttcct ggcccttctg 1080<br>gagtatgcct ttgtcaacta cattttcttt ggaagaggcc ctcaaaggca gaagaagctt 1140<br>gcagaaaaga cagccaaggc aaagaatgac cgttcaaaga gcgaaagcaa ccgggtggat 1200<br>gctcatggaa atattctgtt gacatcgctg gaagttcaca atgaaatgaa tgaggtctca 1260<br>ggcggcattg gcgataccag gaattcagca atatcctttg acaactcagg aatccagtac 1320<br>aggaaacaga gcatgcctcg agaagggcat gggcgattcc tgggggacag aagcctcccg 1380<br>cacaagaaga cccatctacg gaggaggtct tcacagctca aaattaaaat acctgatcta 1440<br>accgatgtga atgccataga cagatggtcc aggatcgtgt ttccattcac tttttctctt 1500<br>ttcaacttag tttactggct gtactatgtt aactgagtga ctgtacttga tttttcaaag 1560<br>acttcattta acactgagtg aaatattact ctgcctgtca agtttttata cctgtacaca 1620<br>cacagacaca caagcagaca cacacatata tacatacgca attgtatata tatgtgaact 1680<br>ttctcagcat atatataaaa tacacgtgta tatgaggatg tatgtgtata tgtttataca 1740<br>cacaggagtc agtgcccatg tgtatggaag acaaatacac atacatatat acattttgca 1800<br>gctatggaca atttaccaca ggatgcatat taaagaaagt catagttttt ttctttttta 1860<br>attgaaaggg acaagtatca tctaaatatt atgccttgag aatgagggcg tgaaacacaa 1920<br>tatcatcccc aaatgtgtct tgtattatca taagttagat gttttagttt aaaaatcaga 1980<br>aagacattct tagttaatct ttgaaaactc atacagtggt attgctagtt taaaatgagt 2040<br>cacttacttc atatcctctc gttcagttta gtaagcaaag gcttcttggc ttctctggtg 2100<br>atggggtttg ttttcatcgg gcatacgttt tctgcaatgg tttagtggct ggggtgagcc 2160<br>actggcagtg tgcttacctg ttgtctgaaa catagataga tcccacgttg atgtctgaac 2220<br>gaccgtcttt tgaaaactca tcgggagtga atggcatctc gttgtaagta ctctaatata 2280<br>cagtgtgtag tttgtttctg ttgttcactt ggagtggatc cagcttcact gtcatgtgcg 2340<br>aacacagtga cacgtttggc cagtgacatt tcaatcactg aaaatgtgct ctacatctcg 2400<br>tatggatttc taggcctgat atccaacaga aagcatagac gtctcaggtt attcgttact 2460<br>ctaaggtaaa accatctagg atgattttcc cccttgcagt tatgttatca ttcttataac 2520<br>attgtatgtt aatagaaaat atatttgcat aatatgcata tatatgtata tttaccaaga 2580<br>ttttgtttct tacgcttgct atcatggcag catgcgatgt catattttcc tttatgtgat 2640<br>gtaactactt tctgttatct agaaattaag attgaagcta aaacacttct actgttcaat 2700<br>ttcagaaact aagaatcatc ctcatgcctt tatttctgta tctgacatat ttcataagca 2760<br>catccaacta ctcctagact gactaggatt ctgcaggaac atgacccgta cacaccacgc 2820<br>gtcacccaac gacccatgac cgttctctga ggcaaaggag ggcaacctga cagcaaacac 2880<br>agtcactgtt ggttccttct gatccacagc ctcatcagta tttggacttt ttaaagctcg 2940<br>tagaaacaag acaaggtgca ccggtttcat agacgcaacc ttaacttact atttagatga 3000<br>gatctttcta aagaaaaaaa aaagagatga tatatttttt gtaaacaata tttctatcac 3060 |

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|---|---|---|
| | | aggcatccat aaactgaaat gactacagtt gtgcaaacag gtgtcacagt gaagttgagc 3120<br>atttggagaa aaaaataaaa agcaaaattt gcaggaagaa ctgctaaatt aatactttat 3180<br>cccaaaatgc cacgtatgcc tcaccctctc tgttctatcc aaaaccaagg accagagtgc 3240<br>tccaatggta ggccccagtt gtctcgatgt aggtagaggc accaccctcc ccgaggatgc 3300<br>gtgtggtgtg gactatcccc atgagctgac cactacttga tttttctttg gtggccgtaa 3360<br>caacctttaa tttgtgggca tctgcaacag ttcaaaaccc accatcagat aaaacataat 3420<br>ccacaaaatc tcacgctaga ggcaattacc tacttttaga ccctttccc tctttcattc 3480<br>ctatcttttt cctcctaatc gctgctctct ggttttattt tcatctggag actagccagg 3540<br>gatttctttg gctttggctt ttctctgacc atttttcca tgggtaacaa tgggggatcc 3600<br>taaaagttaa acagattggg aaaaaccttg ttaagtggcc ttatcattat tatcatgtta 3660<br>aagaaaaaaa atacatttgc aaagacctta tccctttcaa tacttcaggt atctttttct 3720<br>gtatccagta attaaaggtg tgaggtccac atgcagaaga gggaccccaa aatgtaaatg 3780<br>gatgtggaca aaaacagtca atggtctatt taagtgtata atttatacta ttcagaactg 3840<br>tgacattaat tctttctggg agaaaagtga tttaaaactt ttttgatgca tagttgaggt 3900<br>acccaaatat caaaggcaga gaccccatgg ggctccaaag agctgcagtc tccttcccaa 3960<br>ggttttctgg atataaattt gcatggtata gtcataatag cttttgagct ttttatatgc 4020<br>atttggcacc aagactggga tccacaactt tgtagacact gcgatgaagt taacatatta 4080<br>gctatactat aaatagtgtt tgtaactaca cacacacaca cacacacaca cacaaataca 4140<br>tacatatatt ctgtaaaaac aaaaaaaaac tttttaagat ccttgggtat gtgtttgctt 4200<br>tactccattt cagaagaaaa ttacttttct tctaacaaaa ttattttata gcttctccat 4260<br>ttttaaaact tgtgagagca ttgagaagag aactctgact gtgttaacag aagagagttg 4320<br>aattggagtc tctgttgtgt taaaatgacc tctcgttact ccacagtagt tatttgagcc 4380<br>agtgactgag tcgcgttgag gaattctgaa cccggacctc tgacgttgtt gggaggtggc 4440<br>tgttacccac acccagacct cttgagtaag gacagaaacg ttatcattgg ggatataatg 4500<br>agatttcttt cttaacaatt gaaagtaata aagagttaat tttctcagta gtcctgtctt 4560<br>tccaaaatgc gccacagggg ctcaagatct acagaagaat cttgttatga cagtttgtta 4620<br>ttacccatat tcagatatcc ttgagataat ggaagagccc tgctacataa ctccctacag 4680<br>agaaagactg atagacttga gtagtcctta aaacaagtgt tattcctgtt caccaacccc 4740<br>gggactgtgg aggggtttca ctgtctatac catgcgacat ccatttcccc tgaatctcaa 4800<br>acgaactaga aagtatgtca tgataatatt tccattagat ttggaagcta cctgtacatc 4860<br>tgcaatattg tgtttttaac gccagcaccc agaactttga catgttcagt cactccctga 4920<br>aaggcacttc tctcttgtcc aaacacagcg ttgacatttt tactgaggag atcatctcaa 4980<br>aggtgatgcc aaacgagtct ggggctggtt ttaaggggac aggcacattg cagttgtagg 5040<br>tgttcatttt cagcatctag tagataatcc attggtgttt gtcccaccat tggtgtattc 5100<br>taacaagaat gtgtccaact ttgaatctcc tggcttgaaa gtataaacca tcacttaatt 5160<br>cttattttaa ctctccacct gaaaaccagt tcatatttct ggccatttta tgtaagcaaa 5220<br>actgaacaat tggtgaaaca ttttgttact cttggaattg actctggctg tcagtgtgag 5280<br>ccattagagt aacatcgaat cttggggcaa agaactgccc aggtgaatta aatttttcca 5340<br>ggacactagc tagtgtgcct tggattgatt acctcttcta ctgcattgaa aggcgccatg 5400<br>ttttcctgaa atactaaatt cccaacacct gggtaaacaa tgaccttcca gagagtggct 5460<br>cccgtatgcc tctccctagg accaacccca tgaacatgtt ttgtcacgtt tgtctcatgt 5520<br>ttctacttca caagtcagtg agtgtgttta aggtaagtac aggattattc tagtaggaat 5580<br>aggcgattgc tgtcataatc aattctcatg ttgatttcat tttattgtaa agataaattt 5640<br>aaacccagtt ttgcttaagc acattgatgt aatttttgg tattatttga catgaaaaaa 5700<br>cagcaaaatt gagtgataga tac 5723 |
| **3-8** | **Sequences** | |
| 3-8-1 | Sequence Number [ID] | 8 |
| 3-8-2 | Molecule Type | AA |
| 3-8-3 | Length | 473 |
| 3-8-4 | Features Location/ | **source 1..473** |
| | Qualifiers | mol_type=protein |
| | | organism=Homo sapiens |
| | NonEnglishQualifier Value | |
| 3-8-5 | Residues | MWGLAGGRLF GIFSAPVLVA VVCCAQSVND PGNMSFVKET VDKLLKGYDI RLRPDFGGPP 60<br>VCVGMNIDIA SIDMVSEVNM DYTLTMYFQQ YWRDKRLAYS GIPLNLTLDN RVADQLWVPD 120<br>TYFLNDKKSF VHGVTVKNRM IRLHPDGTVL YGLRITTTAA CMMDLRRYPL DEQNCTLEIE 180<br>SYGYTTDDIE FYWRGGDKAV TGVERIELPQ FSIVEHRLVS RNVVFATGAY PRLSLSFRLK 240<br>RNIGYFILQT YMPSILITIL SWVSFWINYD ASAARVALGI TTVLTMTTIN THLRETLPKI 300<br>PYVKAIDMYL MGCFVFVFLA LLEYAFVNYI FFGRGPQRQK KLAEKTAKAK NDRSKSESNR 360<br>VDAHGNILLT SLEVHNEMNE VSGGIGDTRN SAISFDNSGI QYRKQSMPRE GHGRFLGDRS 420<br>LPHKKTHLRR RSSQLKIKIP DLTDVNAIDR WSRIVFPFTF SLFNLVYWLY YVN 473 |
| **3-9** | **Sequences** | |
| 3-9-1 | Sequence Number [ID] | 9 |
| 3-9-2 | Molecule Type | DNA |
| 3-9-3 | Length | 3174 |

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|---|---|---|
| 3-9-4 | Features Location/ | **source 1..3174** |
| | Qualifiers | mol_type=other DNA |
| | | organism=Homo sapiens |
| | NonEnglishQualifier Value | |
| 3-9-5 | Residues | taataatggc cgtaagctta aaatagatcc agggaggagc tcattaacgt gaacatagaa 60<br>agcagttccg cacctctggc cttactcctc ttggaaattg ctttggtcca tttttacttc 120<br>cttttattcg acgcaccaga aaataagact tttaccaaca tttttactgc atttgacgat 180<br>gaactaattt agaccggcta aaataattgt tccactggga cacaggaatt caacctcagt 240<br>tcagaaaatc cctgacatct gacgtaggag gatttatagg tttagtggaa attgctttct 300<br>cctgctctcc agattgcatc ctgtgggttg attttttttt tgcatgagta aacatccttc 360<br>taataatgaa cagaccaata atgtcttaag agagaaaaag aacaatcttt tccttttgc 420<br>tgtttctgga gagagctgtt tgaatttgga aacccatgtt ggctgtccca aatatgagat 480<br>ttggcatctt tcttttgtgg tggggatggg ttttggccac tgaaagcaga atgcactggc 540<br>ccggaagaga agtccacgag atgtctaaga aaggcaggcc ccaaagacaa agacgagaag 600<br>tacatgaaga tgcccacaag caagtcagcc caattctgag acgaagtcct gacatcacca 660<br>aatcgcctct gacaaagtca gaacagcttc tgaggataga tgaccatgat ttcagcatga 720<br>ggcctggctt tggaggccct gccattcctg ttggtgtgga tgtgcaggtg gagagtttgg 780<br>atagcatctc agaggttgac atggacttta cgatgaccct ctacctgagg cactactgga 840<br>aggacgagag gctgtctttt ccaagcacca acaacctcag catgacgttt gacggccggc 900<br>tggtcaagaa gatctgggtc cctgacatgt ttttcgtgca ctccaaacgc tccttcatcc 960<br>acgacaccac cacagacaac gtcatgttgc gggtccagcc tgatgggaaa gtgctctata 1020<br>gtctcagggt tacagtaact gcaatgtgca acatggactt cagccgattt cccttggaca 1080<br>cacaaacgtg ctctcttgaa attgaaagct atgcctatac agaagatgac ctcatgctgt 1140<br>actggaaaaa gggcaatgac tccttaaaga cagatgaacg gatctcactc tcccagttcc 1200<br>tcattcagga attccacacc accaccaaac tggctttcta cagcagcaca ggctggtaca 1260<br>accgtctcta cattaatttc acgttgcgtc gccacatctt cttcttcttg ctccaaactt 1320<br>atttccccgc taccctgatg gtcatgctgt cctgggtgtc cttctggatc gaccgcagag 1380<br>ccgtgcctgc cagagtcccc ttaggtatca caacggtgct gaccatgtcc accatcatca 1440<br>cgggcgtgaa tgcctccatg ccgcgcgtct cctacatcaa ggccgtggac atctacctct 1500<br>gggtcagctt tgtgttcgtg ttcctctcgg tgctggagta tgcggccgtc aactacctga 1560<br>ccactgtgca ggagaggaag gaacagaagc tgcgggagaa gcttccctgc accagcggat 1620<br>tacctccgcc ccgcactgcg atgctggacg gcaactacag tgatggggag gtgaatgacc 1680<br>tggacaacta catgccagag aatggagaga agcccgacag gatgatggtg cagctgaccc 1740<br>tggcctcaga gaggagctcc ccacagagga aaagtcagag aagcagctat gtgagcatga 1800<br>gaatcgacac ccacgccatt gataaatact ccaggatcat ctttccagca gcatacattt 1860<br>tattcaattt aatatactgg tctattttct cctagatgct tgtaattcta caaatttcac 1920<br>atttccatgg catgcactac agaaataact gtataatgaa aaagtattta aggatatggt 1980<br>taaaaaaaaa tcccaggacc cacccatgtt ttcactatcc cttctgcagc tttccaaagc 2040<br>tacattgacg agacacttac tggtttaatt tgcacttatt aaccatctat tgaatacaca 2100<br>gcattatatt aggtgctgca ggaaatacga cactgtagcg actgatgtta gttgttaccc 2160<br>agatcccctg gaaaagcaca ctaccagtgt tgtgggcaca tttagttcca cccgttagac 2220<br>ccttgatgct attcacatga ataatttatt ttcctcaagt gtcattacat tgttcaggct 2280<br>acgtgaactt ggaagcacct acaggccatt tgcatgaaat tcacatgcac ctaaatcctc 2340<br>actttgacag aaactcatgc ttcagtttat aacctattac ctattttgta tgcgactcca 2400<br>cctccgcatg tttattttaa taaaaggcaa tgataacatt cacattattt ttctttatat 2460<br>gctgtggttc acaggcttta ccccttcaca agaaaagctc tttagattgg cgcaattgct 2520<br>tctgattttg gtgaaatttt ccctggtagg gaaactttga agataagagt acacacatgc 2580<br>attttgtctg ttgtgtcata gaggtaacta ggctagaaaa tttgtgttta aatgttccct 2640<br>attttatata atcaccactt catgtttctt cttcttggag catgtccttg ttcaaagaga 2700<br>agtgctttct cagtgatgtg atatcttcac tgaggaactt gggtagagaa tgatttcttc 2760<br>tgcataaaca cttcaaggaa atacataatt tgggactact tgtaactcat tagaatgaga 2820<br>aatactcaca tggtttctta agagaaaaag aacatcggaa agcaaaataa atgggaagat 2880<br>atcactggac atctgcattt atactcgaaa taccagcatt ttctatggac cagaaaactg 2940<br>ccatcaccta gaccacacag cccagatacc aggcagacgg atggcccaat ggcaactgat 3000<br>gtcagggcat ggggtaaagg agagggttct aatctggtgt atcacttaaa aacagttatt 3060<br>tatattatat atctgctata tagatcaacc tccaccaaac ttacccaaac agcatttgtt 3120<br>ttatttgaaa ctcactttaa taaagtgaat tatatacaca aaaaaaaaaa aaaa 3174 |
| **3-10** | **Sequences** | |
| 3-10-1 | Sequence Number [ID] | 10 |
| 3-10-2 | Molecule Type | AA |
| 3-10-3 | Length | 479 |
| 3-10-4 | Features Location/ | **source 1..479** |
| | Qualifiers | mol_type=protein |
| | | organism=Homo sapiens |
| | NonEnglishQualifier Value | |

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|---|---|---|
| 3-10-5 | Residues | MLAVPNMRFG IFLLWWGWVL ATESRMHWPG REVHEMSKKG RPQRQRREVH EDAHKQVSPI 60<br>LRRSPDITKS PLTKSEQLLR IDDHDFSMRP GFGGPAIPVG VDVQVESLDS ISEVDMDFTM 120<br>TLYLRHYWKD ERLSFPSTNN LSMTFDGRLV KKIWVPDMFF VHSKRSFIHD TTTDNVMLRV 180<br>QPDGKVLYSL RVTVTAMCNM DFSRFPLDTQ TCSLEIESYA YTEDDLMLYW KKGNDSLKTD 240<br>ERISLSQFLI QEFHTTTKLA FYSSTGWYNR LYINFTLRRH IFFFLLQTYF PATLMVMLSW 300<br>VSFWIDRRAV PARVPLGITT VLTMSTIITG VNASMPRVSY IKAVDIYLWV SFVFVFLSVL 360<br>EYAAVNYLTT VQERKEQKLR EKLPCTSGLP PPRTAMLDGN YSDGEVNDLD NYMPENGEKP 420<br>DRMMVQLTLA SERSSPQRKS QRSSYVSMRI DTHAIDKYSR IIFPAAYILF NLIYWSIFS 479 |
| **3-11** | **Sequences** | |
| 3-11-1 | Sequence Number [ID] | 11 |
| 3-11-2 | Molecule Type | DNA |
| 3-11-3 | Length | 4620 |
| 3-11-4 | Features Location/ | **source 1..4620** |
| | Qualifiers | mol_type=other DNA |
| | | organism=Homo sapiens |
| | NonEnglishQualifier Value | |
| 3-11-5 | Residues | atgccttatt ttacaagact cattttgttc ttgttttgct tgatggttct cgtggagagc 60<br>agaaaaccca agaggaagcg atggacaggg caggtggaaa tgcccaagcc aagtcactta 120<br>tataagaaga accttgatgt gaccaagatc cggaagggaa agcctcagca gcttctcaga 180<br>gtggacgagc acgacttcag catgagaccc gccttcggag gccctgccat cccggtgggc 240<br>gtggacgtac aggtggagag cctggacagc atctccgagg tggacatgga cttcactatg 300<br>accctgtacc tgcggcatta ctggaaggat gagaggctag ctttctccag cgccagcaac 360<br>aagagcatga ccttcgatgg ccggctggtg aagaagatct gggtccctga tgtcttcttt 420<br>gttcactcca aaagatcgtt cactcatgac accaccactg acaacatcat gctgagggtg 480<br>ttcccagatg gacacgtgct gtacagcatg aggattacgg tcactgccat gtgcaacatg 540<br>gacttcagcc actttcccct ggactcccag acctgttctt tggagctgga gagctatgcc 600<br>tatacagatg aagatctaat gctgtactgg aagaatgggg atgaatccct aaaaacagat 660<br>gagaagatct ccttgtctca gtttctgatt cagaaatttc acacaacttc caggctggcc 720<br>ttctacagca gcactggctg gtacaaccgt ctgtacatta acttcacgtt gcgtcgccac 780<br>atcttcttct tcttgctcca aacatatttc cctgccactc tgatggtcat gctgtcctgg 840<br>gtgtccttct ggatcgaccg cagagctgtg cctgccagag tttcactggg tatcacgacg 900<br>gtgctgacca tgaccaccat catcacgggc gtgaatgcct ccatgccgcg cgtctcctac 960<br>gtcaaggccg tggacatcta cctctgggtc agctttgtgt tcgtgttcct ctcggtgctg 1020<br>gagtatgcgg ctgtcaacta cctgaccacc gtgcaggagc gcaaggaacg gaagctgcgg 1080<br>gagaagttcc cgtgcatgtg tggaatgctt cattcaaaaa ccatgatgct ggatggaagc 1140<br>tacagtgagt ctgaggccaa cagcctggct gggtacccca gaagccatat cctgacagaa 1200<br>gaagaaaggc aagacaaaat agtggtccac ctgggcctga gtggtgaagc caacgctgcc 1260<br>agaaagaagg ggcttctgaa gggccagacg ggttttcgta tcttccagaa tacccatgcc 1320<br>attgacaaat actctaggtt gatattccct gcctcctaca tatttttcaa cttaatttat 1380<br>tggtcagtgt tttcctaggg gctccaaggc tgttcctaga agagggcata gacatcgagg 1440<br>gggcctggcc agtcattgac agacggactt gttgaccaca cgcccctcac caaacaatgc 1500<br>agcagctact ggaccaccct gagcagcact catctctcag agaagcccag gagccttcca 1560<br>gctgccctga ccccagaccc cgtggggctg ctccatgttc atgctgtctc gcgtcacact 1620<br>tcacatctct ctgggacctt ctgttcttgt gtgtgaacta attcacaaga actcccctcc 1680<br>tataaacaag gattcaaatg cctcctagac attcttagac cctcagattg cctaggagtc 1740<br>agttgtggag ggaaaaggaa aaactgaagt acaagcttag gggcattctg gataggagat 1800<br>aggaaattaa gaacaaaaaa caccctgtaa acccattgaa cttgattaag tgcctaccta 1860<br>gaagggaaaa gagctgagat cccagacagg aaatgatttg gcctgtggga gtatggcaac 1920<br>ccacaggatt ctgcaatatt agaggagatt attcatttat ccatccattc aaccattcat 1980<br>taaatattga ggtcccactc tgtgccaggt acggtagaaa tgagaaaata gtctctttcc 2040<br>ttagagatct tctctaagtt gtgctcattc tacatatggc atgatgtact tctgctcttt 2100<br>cccctccttc aaccccgctt accccacaga cccattctgt ttgctgttgc ttttactctt 2160<br>aggcatataa ggcagggtcc aggagaggcc aggtccagaa ctttcaacta tcctcctcca 2220<br>gtgaagttac acagatagca ctaattttgc ccagcaatga catgtagcaa tgtgcatgga 2280<br>gtattgccag ccagagaaac ttacccaggc tcaccacggt gtacagtttt tttattgggg 2340<br>tcggtcatgg atttatagct gattgcccac atggctgact ttagtctttt gcccctccag 2400<br>aggttaagct ggtaccttga ggtccagggc acctatcata aatcacattg ttggcataga 2460<br>ctatctggtg tggcccaagg ccccagtaga caaagaccct tctatctcac aggacattcc 2520<br>aagggcatag aggttgcttc ctagtaaagg ttagtccttt actacacagc aactcttgtc 2580<br>aattccctat tgttaatgtt aattgcggtg ttactattat atattcaggc aataaacatt 2640<br>ttaaagtatc tctgcactag gtacattggg gtccaaatta agaataagag tagtcgaagc 2700<br>tctcagagaa ggaactagta aaggaaatac cacacaaata tctataatat aagattaaga 2760<br>gtgtttagtg cctcagtaat agttcagtgt gagatgggaa ttccaaggga ggaaaaacag 2820<br>tcccaccagg gagacagacc tcatggagaa agcagccctg agatgcccct tgaaggtcag 2880<br>gattttaaga agaagaaatg gagcagaatg catcctagat ctgggctgtc tcatatggta 2940 |

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| | | gccactagcc acatgtctat ttaaatttaa attagtaaaa attaaaaata ttagccaggt 3000<br>gtggtggtgt gtgcctgggg ttctagctac tcaggaggct gaggcgggag gattgcttga 3060<br>gcccaggaat tccaggctgc cgtgagctgt gatcaagcca ctgcagtcca gcctgagtga 3120<br>cagagcgaga ccctatctct aaaataataa ttattattat ttaaattagt tgaagttaaa 3180<br>taaaattgaa aactcagttc cttggttaca ccatccacac tcaagtgggc ttagtaagca 3240<br>cagttgctag tagctactgc actgggctgg gcagcctaaa gaccaggaag ctctaccagc 3300<br>caagagacca gcttgagcgg acgcatgggg agaatgggaa ggtttgccat tgcagagaac 3360<br>agggtttgtg ggcaagtcat tcattttggc aggatcctag gctgtgataa gaggaggtga 3420<br>gcgactagct ttttccacaa cactagggat caaggcaggt ccccaccatg ccggtgctta 3480<br>tgaggacagc ttccccacta tggagttgct ggacccaaga ctactagatt tcccagtctc 3540<br>acagaccaat gaagaacaaa aatgcataaa tatgggtaat tgggtggtac agggcatctg 3600<br>gacttgttta gaaacctttt tttcttttct gagacagagt ttcactcttg ttgctcaggc 3660<br>tggagtgcaa tggcttgatc ttggctcacc acaacctctg cctcccgggt tcaagcaatt 3720<br>ctcctgcctc agccctggag tagctgggat tacaggcatg cgccaccatg cctggctaac 3780<br>ttttgtattt tttagtagag atggggtttc tccatgttag tcagcctggt cttgaactcc 3840<br>tgacctcagg tgatccgcct gcctcagcct cccaaagtgc tgtgattaca ggcgtgagcc 3900<br>accacacccg gccagaaacc ttttatctat agagactctc ccaggccaca gtgctgctcg 3960<br>aagaatgata attctattgt cattaaatat atcatattgc tttgcttgta agtcttattt 4020<br>aattccaacc cttctctgct tctgactgca ttgttttcat gcattatggc tcatataaca 4080<br>tagttgcctg ttcacaaatt aaaacaaata tttatttttg ctagcttcta tgttaaactg 4140<br>gagttaattc tatagcagac atatttagaa tcataaacag tgttttatca tgcagggcca 4200<br>tgatacaatg acaccattgt atttagcatt gccttattac agtatactag tctgacacta 4260<br>cttgagaaaa gtaggaaatt taaagaggta aagtggagac aacattggta ggagtagaaa 4320<br>acaggaacta aggaaatagt gagaacatgg gatgattcac cctggaggca ggaagacaga 4380<br>gaagcaaaca caattgttat caggaatgca tgaatgatac tttacttcac tgaatctaag 4440<br>acatggtgaa atgtaagatg cacccatatt ttatgtactg agaaaaaata ctaacaatca 4500<br>aattctggaa tgccaatgat tataatatga atcctgattt caccaatatg aaactgtgaa 4560<br>aaaatgttaa tgttacaata aaagaaatgg gttttataca aaaaaaaaaa aaaaaaaaaa 4620 |
| **3-12** | **Sequences** | |
| 3-12-1 | Sequence Number [ID] | 12 |
| 3-12-2 | Molecule Type | AA |
| 3-12-3 | Length | 465 |
| 3-12-4 | Features Location/ | **source 1..465** |
| | Qualifiers | mol_type=protein |
| | | organism=Homo sapiens |
| | NonEnglishQualifier Value | |
| 3-12-5 | Residues | MPYFTRLILF LFCLMVLVES RKPKRKRWTG QVEMPKPSHL YKKNLDVTKI RKGKPQQLLR 60<br>VDEHDFSMRP AFGGPAIPVG VDVQVESLDS ISEVDMDFTM TLYLRHYWKD ERLAFSSASN 120<br>KSMTFDGRLV KKIWVPDVFF VHSKRSFTHD TTTDNIMLRV FPDGHVLYSM RITVTAMCNM 180<br>DFSHFPLDSQ TCSLELESYA YTDEDLMLYW KNGDESLKTD EKISLSQFLI QKFHTTSRLA 240<br>FYSSTGWYNR LYINFTLRRH IFFFLLQTYF PATLMVMLSW VSFWIDRRAV PARVSLGITT 300<br>VLTMTTIITG VNASMPRVSY VKAVDIYLWV SFVFVFLSVL EYAAVNYLTT VQERKERKLR 360<br>EKFPCMCGML HSKTMMLDGS YSESEANSLA GYPRSHILTE EERQDKIVVH LGLSGEANAA 420<br>RKKGLLKGQT GFRIFQNTHA IDKYSRLIFP ASYIFFNLIY WSVFS 465 |
| **3-13** | **Sequences** | |
| 3-13-1 | Sequence Number [ID] | 13 |
| 3-13-2 | Molecule Type | DNA |
| 3-13-3 | Length | 1404 |
| 3-13-4 | Features Location/ | **source 1..1404** |
| | Qualifiers | mol_type=other DNA |
| | | organism=Homo sapiens |
| | NonEnglishQualifier Value | |
| 3-13-5 | Residues | atggtcctgg ctttccagtt agtctccttc acctacatct ggatcatatt gaaaccaaat 60<br>gtttgtgctg cttctaacat caagatgaca caccagcggt gctcctcttc aatgaaacaa 120<br>acctgcaaac aagaaactag aatgaagaaa gatgacagta ccaaagcgcg gcctcagaaa 180<br>tatgagcaac ttctccatat agaggacaac gatttcgcaa tgagacctgg atttggaggg 240<br>tctccagtgc cagtaggtat agatgtccat gttgaaagca ttgacagcat ttcagagact 300<br>aacatggact ttacaatgac tttttatctc aggcattact ggaaagacga gaggctctcc 360<br>tttcctagca cagcaaacaa aagcatgaca tttgatcata gattgaccag aaagatctgg 420<br>gtgcctgata tctttttttgt ccactctaaa agatccttca tccatgatac aactatggag 480<br>aatatcatgc tgcgcgtaca ccctgatgga aacgtcctcc taagtctcag gataacggtt 540<br>tcggccatgt gctttatgga tttcagcagg tttcctcttg acactcaaaa ttgttctctt 600<br>gaactggaaa gctatgccta caatgaggat gacctaatgc tatactggaa acacggaaac 660<br>aagtccttaa atactgaaga acatatgtcc ctttctcagt tcttcattga agacttcagt 720<br>gcatctagtg gattagcttt ctatagcagc acaggttggt acaataggct tttcatcaac 780 |

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| | | tttgtgctaa ggaggcatgt tttcttcttt gtgctgcaaa cctatttccc agccatattg 840<br>atggtgatgc tttcatgggt ttcattttgg attgaccgaa gagctgttcc tgcaagagtt 900<br>tccctgggaa tcaccacagt gctgaccatg tccacaatca tcactgctgt gagcgcctcc 960<br>atgccccagg tgtcctacct caaggctgtg gatgtgtacc tgtgggtcag ctcctcttt 1020<br>gtgttcctgt cagtcattga gtatgcagct gtgaactacc tcaccacagt ggaagagcgg 1080<br>aaacaattca agaagacagg aaagatttct aggatgtaca atattgatgc agttcaagct 1140<br>atggcctttg atggttgtta ccatgacagc gagattgaca tggaccagac ttcctctct 1200<br>ctaaactcag aagacttcat gagaagaaaa tcgatatgca gccccagcac cgattcatct 1260<br>cggataaaga gaagaaaatc cctaggagga catgttggta gaatcattct ggaaaacaac 1320<br>catgtcattg acacctattc taggatttta ttccccattg tgtatatttt atttaatttg 1380<br>ttttactggg gtgtatatgt atga 1404 |
| **3-14** | **Sequences** | |
| 3-14-1 | Sequence Number [ID] | 14 |
| 3-14-2 | Molecule Type | AA |
| 3-14-3 | Length | 467 |
| 3-14-4 | Features Location/ | **source 1..467** |
| | Qualifiers | mol_type=protein |
| | | organism=Homo sapiens |
| | NonEnglishQualifier Value | |
| 3-14-5 | Residues | MVLAFQLVSF TYIWIILKPN VCAASNIKMT HQRCSSSMKQ TCKQETRMKK DDSTKARPQK 60<br>YEQLLHIEDN DFAMRPGFGG SPVPVGIDVH VESIDSISET NMDFTMTFYL RHYWKDERLS 120<br>FPSTANKSMT FDHRLTRKIW VPDIFFVHSK RSFIHDTTME NIMLRVHPDG NVLLSLRITV 180<br>SAMCFMDFSR FPLDTQNCSL ELESYAYNED DLMLYWKHGN KSLNTEEHMS LSQFFIEDFS 240<br>ASSGLAFYSS TGWYNRLFIN FVLRRHVFFF VLQTYFPAIL MVMLSWVSFW IDRRAVPARV 300<br>SLGITTVLTM STIITAVSAS MPQVSYLKAV DVYLWVSSLF VFLSVIEYAA VNYLTTVEER 360<br>KQFKKTGKIS RMYNIDAVQA MAFDGCYHDS EIDMDQTSLS LNSEDFMRRK SICSPSTDSS 420<br>RIKRRKSLGG HVGRIILENN HVIDTYSRIL FPIVYILFNL FYWGVYV 467 |
| **3-15** | **Sequences** | |
| 3-15-1 | Sequence Number [ID] | 15 |
| 3-15-2 | Molecule Type | DNA |
| 3-15-3 | Length | 1320 |
| 3-15-4 | Features Location/ | **misc_feature 1..1320** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..1320** |
| | | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-15-5 | Residues | atgcgctgct cgccgggagg cgtctggctg gcgctggccg cgtcgctcct gcacgtgtcc 60<br>ctgcaaggcg agttccagag gaagctttac aaggagctgg tcaagaacta caatcccttg 120<br>gagaggcccg tggccaatga ctcgcaacca ctcaccgtct acttctccct gagcctcctg 180<br>cagatcatgg acgtggatga gaagaaccaa gttttaacca ccaacatttg gctgcaaatg 240<br>tcttggacag atcactattt acagtggaat gtgtcagaat atccaggggt gaagactgtt 300<br>cgtttcccag atggccagat ttggaaacca gacattcttc tctataacag tgctgatgag 360<br>cgctttgacg ccacattcca cactaacgtg ttggtgaact cttctgggca ttgccagtac 420<br>ctgcctccag gcatattcaa gagttcctgc tacatcgatg tacgctggtt tccctttgat 480<br>gtgcagcact gcaaactgaa gtttgggtcc tggtcttacg gaggctggtc cttggatctg 540<br>cagatgcagg aggcagatat cagtggctat atccccaatg gagaatggga cctagtggga 600<br>atccccggca agaggagtga aaggttctat gagtgctgca aagagcccta ccccgatgtc 660<br>accttcacag tgaccatgcg ccgcaggatg ggttactacc tgattcagat gtatattccc 720<br>agcctgctca ttgtcatcct ctcatggatc tccttctgga tcaacatgga tgctgcacct 780<br>gctcgtgtgg gcctaggcat caccactgtg ctcaccatga ccacccagag ctccggctct 840<br>cgagcatctc tgcccaaggt gtcctatgtg aaagccattg acatttggat ggcagtttgc 900<br>ctgctctttg tgttctcagc cctattagaa tatgctgccg ttaactttgt gtctcggcaa 960<br>cataaggagc tgctccgatt caggaggaag cggagacatc acaagagccc catgttgaat 1020<br>ctattccagg aggatgaagc tggagaaggc cgctttaact tctctgccta tgggatgggc 1080<br>ccagcctgtc tacaggccaa ggatggcatc tcagtcaagg gcgccaacaa cagtaacacc 1140<br>accaaccccc ctcctgcacc atctaagtcc ccagaggaga tgcgaaaact cttcatccag 1200<br>agggccaaga agatcgacaa aatatcccgc attggcttcc ccatggcctt cctcattttc 1260<br>aacatgttct actggatcat ctacaagatt gtccgtagag aggacgtcca caaccagtga 1320 |
| **3-16** | **Sequences** | |
| 3-16-1 | Sequence Number [ID] | 16 |
| 3-16-2 | Molecule Type | AA |
| 3-16-3 | Length | 439 |
| 3-16-4 | Features Location/ | **REGION 1..439** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |

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|---|---|---|
| | | **source 1..439** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-16-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDEKNQ VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV RFPDGQIWKP DILLYNSADE 120<br>RFDATFHTNV LVNSSGHCQY LPPGIFKSSC YIDVRWFPFD VQHCKLKFGS WSYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFY ECCKEPYPDV TFTVTMRRRM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS RASLPKVSYV KAIDIWMAVC 300<br>LLFVFSALLE YAAVNFVSRQ HKELLRFRRK RRHHKSPMLN LFQEDEAGEG RFNFSAYGMG 360<br>PACLQAKDGI SVKGANNSNT TNPPPAPSKS PEEMRKLFIQ RAKKIDKISR IGFPMAFLIF 420<br>NMFYWIIYKI VRREDVHNQ 439 |
| **3-17** | **Sequences** | |
| 3-17-1 | Sequence Number [ID] | 17 |
| 3-17-2 | Molecule Type | AA |
| 3-17-3 | Length | 439 |
| 3-17-4 | Features Location/ | **REGION 1..439** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..439** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-17-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDEKNQ VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV RFPDGQIWKP DILLYNSADE 120<br>RFDATFHTNV LVNSSGHCQY LPPGIFKSSC YIDVRWFPFD VQHCKLKFGS WSYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFY ECCKEPYPDV TFTVTMRRQM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS RASLPKVSYV KAIDIWMAVC 300<br>LLFVFSALLE YAAVNFVSRQ HKELLRFRRK RRHHKSPMLN LFQEDEAGEG RFNFSAYGMG 360<br>PACLQAKDGI SVKGANNSNT TNPPPAPSKS PEEMRKLFIQ RAKKIDKISR IGFPMAFLIF 420<br>NMFYWIIYKI VRREDVHNQ 439 |
| **3-18** | **Sequences** | |
| 3-18-1 | Sequence Number [ID] | 18 |
| 3-18-2 | Molecule Type | AA |
| 3-18-3 | Length | 439 |
| 3-18-4 | Features Location/ | **REGION 1..439** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..439** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-18-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDEKNQ VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV RFPDGQIWKP DILLYNSADE 120<br>RFDATFHTNV LVNSSGHCQY LPPGIFKSSC YIDVRWFPFD VQHCKLKFGS WSYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFY ECCKEPYPDV TFTVHLERQM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS RASLPKVSYV KAIDIWMAVC 300<br>LLFVFSALLE YAAVNFVSRQ HKELLRFRRK RRHHKSPMLN LFQEDEAGEG RFNFSAYGMG 360<br>PACLQAKDGI SVKGANNSNT TNPPPAPSKS PEEMRKLFIQ RAKKIDKISR IGFPMAFLIF 420<br>NMFYWIIYKI VRREDVHNQ 439 |
| **3-19** | **Sequences** | |
| 3-19-1 | Sequence Number [ID] | 19 |
| 3-19-2 | Molecule Type | AA |
| 3-19-3 | Length | 439 |
| 3-19-4 | Features Location/ | **REGION 1..439** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..439** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-19-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDEKNQ VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV RFPDGQIWKP DILLYNSADE 120<br>RFDATFHTNV LVNSSGHCQY LPPGIFKSSC YIDVRWFPFD VQHCKLKFGS WSYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFY ECCKEPYPDV TFTVTMRRRT LYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS RASLPKVSYV KAIDIWMAVC 300<br>LLFVFSALLE YAAVNFVSRQ HKELLRFRRK RRHHKSPMLN LFQEDEAGEG RFNFSAYGMG 360 |

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| | | PACLQAKDGI SVKGANNSNT TNPPPAPSKS PEEMRKLFIQ RAKKIDKISR IGFPMAFLIF 420<br>NMFYWIIYKI VRREDVHNQ 439 |
| **3-20** | **Sequences** | |
| 3-20-1 | Sequence Number [ID] | 20 |
| 3-20-2 | Molecule Type | DNA |
| 3-20-3 | Length | 1329 |
| 3-20-4 | Features Location/ | **misc_feature 1..1329** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..1329** |
| | | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-20-5 | Residues | atgcgctgct cgccgggagg cgtctggctg gcgctggccg cgtcgctcct gcacgtgtcc 60<br>ctgcaaggcg agttccagag gaagctttac aaggagctgg tcaagaacta caatcccttg 120<br>gagaggcccg tggccaatga ctcgcaacca ctcaccgtct acttctccct gagcctcctg 180<br>cagatcatgg acgtggatga gaagaaccaa gttttaacca ccaacatttg gctgcaaatg 240<br>tcttggacag atcactattt acagtggaat gtgtcagaat atccaggggt gaagactgtt 300<br>cgtttcccag atggccagat ttggaaacca gacattcttc tctataacag tgctgatgag 360<br>cgctttgacg ccacattcca cactaacgtg ttggtgaact cttctgggca ttgccagtac 420<br>ctgcctccag gcatattcaa gagttcctgc tacatcgatg tacgctggtt tccctttgat 480<br>gtgcagcact gcaaactgaa gtttgggtcc tggtcttacg gaggctggtc cttggatctg 540<br>cagatgcagg aggcagatat cagtggctat atccccaatg gagaatggga cctagtggga 600<br>atccccggca agaggagtga aaggttctat gagtgctgca aagagcccta ccccgatgtc 660<br>accttcacag tgaccatgcg ccgcaggatg ggttactacc tgattcagat gtatattccc 720<br>agcctgctca ttgtcatcct ctcatggatc tccttctgga tcaacatgga tgctgcacct 780<br>gctcgtgtgg gcctaggcat caccactgtg ctcaccatga ccacccagag ctccggctct 840<br>gagatcatgc ccgcaacatc cgattcggta tcctatgtga aagccattga catttggatg 900<br>gcagtttgcc tgctctttgt gttctcagcc ctattagaat atgctgccgt taactttgtg 960<br>tctcggcaac ataaggagct gctccgattc aggaggaagc ggagacatca caagagcccc 1020<br>atgttgaatc tattccagga ggatgaagct ggagaaggcc gctttaactt ctctgcctat 1080<br>gggatgggcc cagcctgtct acaggccaag gatggcatct cagtcaaggg cgccaacaac 1140<br>agtaacacca ccaacccccc tcctgcacca tctaagtccc cagaggagat gcgaaaactc 1200<br>ttcatccaga gggccaagaa gatcgacaaa atatcccgca ttggcttccc catggccttc 1260<br>ctcattttca acatgttcta ctggatcatc tacaagattg tccgtagaga ggacgtccac 1320<br>aaccagtga 1329 |
| **3-21** | **Sequences** | |
| 3-21-1 | Sequence Number [ID] | 21 |
| 3-21-2 | Molecule Type | AA |
| 3-21-3 | Length | 442 |
| 3-21-4 | Features Location/ | **REGION 1..442** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..442** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-21-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDEKNQ VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV RFPDGQIWKP DILLYNSADE 120<br>RFDATFHTNV LVNSSGHCQY LPPGIFKSSC YIDVRWFPFD VQHCKLKFGS WSYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFY ECCKEPYPDV TFTVTMRRRM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS EIMPATSDSV SYVKAIDIWM 300<br>AVCLLFVFSA LLEYAAVNFV SRQHKELLRF RRKRRHHKSP MLNLFQEDEA GEGRFNFSAY 360<br>GMGPACLQAK DGISVKGANN SNTTNPPPAP SKSPEEMRKL FIQRAKKIDK ISRIGFPMAF 420<br>LIFNMFYWII YKIVRREDVH NQ 442 |
| **3-22** | **Sequences** | |
| 3-22-1 | Sequence Number [ID] | 22 |
| 3-22-2 | Molecule Type | DNA |
| 3-22-3 | Length | 1332 |
| 3-22-4 | Features Location/ | **misc_feature 1..1332** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..1332** |
| | | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-22-5 | Residues | atgcgctgct cgccgggagg cgtctggctg gcgctggccg cgtcgctcct gcacgtgtcc 60<br>ctgcaaggcg agttccagag gaagctttac aaggagctgg tcaagaacta caatcccttg 120 |

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| | | gagaggcccg tggccaatga ctcgcaacca ctcaccgtct acttctccct gagcctcctg 180<br>cagatcatgg acgtggatga gaagaaccaa gttttaacca ccaacatttg gctgcaaatg 240<br>tcttggacag atcactattt acagtggaat gtgtcagaat atccaggggt gaagactgtt 300<br>cgtttcccag atggccagat ttggaaacca gacattcttc tctataacag tgctgatgag 360<br>cgctttgacg ccacattcca cactaacgtg ttggtgaact cttctgggca ttgccagtac 420<br>ctgcctccag gcatattcaa gagttcctgc tacatcgatg tacgctggtt tccctttgat 480<br>gtgcagcact gcaaactgaa gtttgggtcc tggtcttacg gaggctggtc cttggatctg 540<br>cagatgcagg aggcagatat cagtggctat atccccaatg gagaatggga cctagtggga 600<br>atccccggca agaggagtga aaggttctat gagtgctgca aagagcccta ccccgatgtc 660<br>accttcacag tgaccatgcg ccgcaggatg ggttactacc tgattcagat gtatattccc 720<br>agcctgctca ttgtcatcct ctcatggatc tccttctgga tcaacatgga tgctgcacct 780<br>gctcgtgtgg gcctaggcat caccactgtg ctcaccatga ccacccagag ctccggctct 840<br>gagatcatgc ccgcaacatc cgattcggta ccattgatag cccaggccat tgacatttgg 900<br>atggcagttt gcctgctctt tgtgttctca gccctattag aatatgctgc cgttaacttt 960<br>gtgtctcggc aacataagga gctgctccga ttcaggagga agcggagaca tcacaagagc 1020<br>cccatgttga atctattcca ggaggatgaa gctggagaag gccgctttaa cttctctgcc 1080<br>tatgggatgg gcccagcctg tctacaggcc aaggatggca tctcagtcaa gggcgccaac 1140<br>aacagtaaca ccaccaaccc ccctcctgca ccatctaagt ccccagagga gatgcgaaaa 1200<br>ctcttcatcc agagggccaa gaagatcgac aaaatatccc gcattggctt ccccatggcc 1260<br>ttcctcattt tcaacatgtt ctactggatc atctacaaga ttgtccgtag agaggacgtc 1320<br>cacaaccagt ga 1332 |
| **3-23** | **Sequences** | |
| 3-23-1 | Sequence Number [ID] | 23 |
| 3-23-2 | Molecule Type | AA |
| 3-23-3 | Length | 443 |
| 3-23-4 | Features Location/ | **REGION 1..443** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..443** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-23-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDEKNQ VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV RFPDGQIWKP DILLYNSADE 120<br>RFDATFHTNV LVNSSGHCQY LPPGIFKSSC YIDVRWFPFD VQHCKLKFGS WSYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFY ECCKEPYPDV TFTVTMRRRM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS EIMPATSDSV PLIAQAIDIW 300<br>MAVCLLFVFS ALLEYAAVNF VSRQHKELLR FRRKRRHHKS PMLNLFQEDE AGEGRFNFSA 360<br>YGMGPACLQA KDGISVKGAN NSNTTNPPPA PSKSPEEMRK LFIQRAKKID KISRIGFPMA 420<br>FLIFNMFYWI IYKIVRREDV HNQ 443 |
| **3-24** | **Sequences** | |
| 3-24-1 | Sequence Number [ID] | 24 |
| 3-24-2 | Molecule Type | DNA |
| 3-24-3 | Length | 1323 |
| 3-24-4 | Features Location/ | **misc_feature 1..1323** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..1323** |
| | | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-24-5 | Residues | atgcgctgct cgccgggagg cgtctggctg gcgctggccg cgtcgctcct gcacgtgtcc 60<br>ctgcaaggcg agttccagag gaagctttac aaggagctgg tcaagaacta caatcccttg 120<br>gagaggcccg tggccaatga ctcgcaacca ctcaccgtct acttctccct gagcctcctg 180<br>cagatcatgg acgtggatga gaagaaccaa gttttaacca ccaacatttg gctgcaaatg 240<br>tcttggacag atcactattt acagtggaat gtgtcagaat atccaggggt gaagactgtt 300<br>cgtttcccag atggccagat ttggaaacca gacattcttc tctataacag tgctgatgag 360<br>cgctttgacg ccacattcca cactaacgtg ttggtgaact cttctgggca ttgccagtac 420<br>ctgcctccag gcatattcaa gagttcctgc tacatcgatg tacgctggtt tccctttgat 480<br>gtgcagcact gcaaactgaa gtttgggtcc tggtcttacg gaggctggtc cttggatctg 540<br>cagatgcagg aggcagatat cagtggctat atccccaatg gagaatggga cctagtggga 600<br>atccccggca agaggagtga aaggttctat gagtgctgca aagagcccta ccccgatgtc 660<br>accttcacag tgaccatgcg ccgcaggatg ggttactacc tgattcagat gtatattccc 720<br>agcctgctca ttgtcatcct ctcatggatc tccttctgga tcaacatgga tgctgcacct 780<br>gctcgtgtgg gcctaggcat caccactgtg ctcaccatga ccacccagag ctccggctct 840<br>cgagcatctc tgcccaaggt gtccgattcg gtaccattga ttgacatttg gatggcagtt 900<br>tgcctgctct ttgtgttctc agccctatta gaatatgctg ccgttaactt tgtgtctcgg 960 |

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| | | caacataagg agctgctccg attcaggagg aagcggagac atcacaagag ccccatgttg 1020<br>aatctattcc aggaggatga agctggagaa ggccgcttta acttctctgc ctatgggatg 1080<br>ggcccagcct gtctacaggc caaggatggc atctcagtca agggcgccaa caacagtaac 1140<br>accaccaacc cccctcctgc accatctaag tccccagagg agatgcgaaa actcttcatc 1200<br>cagagggcca agaagatcga caaaatatcc cgcattggct tccccatggc cttcctcatt 1260<br>ttcaacatgt tctactggat catctacaag attgtccgta gagaggacgt ccacaaccag 1320<br>tga 1323 |
| **3-25** | **Sequences** | |
| 3-25-1 | Sequence Number [ID] | 25 |
| 3-25-2 | Molecule Type | AA |
| 3-25-3 | Length | 440 |
| 3-25-4 | Features Location/ | **REGION 1..440** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..440** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-25-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDEKNQ VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV RFPDGQIWKP DILLYNSADE 120<br>RFDATFHTNV LVNSSGHCQY LPPGIFKSSC YIDVRWFPFD VQHCKLKFGS WSYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFY ECCKEPYPDV TFTVTMRRRM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS RASLPKVSDS VPLIDIWMAV 300<br>CLLFVFSALL EYAAVNFVSR QHKELLRFRR KRRHHKSPML NLFQEDEAGE GRFNFSAYGM 360<br>GPACLQAKDG ISVKGANNSN TTNPPPAPSK SPEEMRKLFI QRAKKIDKIS RIGFPMAFLI 420<br>FNMFYWIIYK IVRREDVHNQ 440 |
| **3-26** | **Sequences** | |
| 3-26-1 | Sequence Number [ID] | 26 |
| 3-26-2 | Molecule Type | DNA |
| 3-26-3 | Length | 1332 |
| 3-26-4 | Features Location/ | **misc_feature 1..1332** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..1332** |
| | | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-26-5 | Residues | atgcgctgct cgccgggagg cgtctggctg gcgctggccg cgtcgctcct gcacgtgtcc 60<br>ctgcaaggcg agttccagag gaagctttac aaggagctgg tcaagaacta caatcccttg 120<br>gagaggcccg tggccaatga ctcgcaacca ctcaccgtct acttctccct gagcctcctg 180<br>cagatcatgg acgtggatga gaagaaccaa gttttaacca ccaacatttg gctgcaaatg 240<br>tcttggacag atcactattt acagtggaat gtgtcagaat atccaggggt gaagactgtt 300<br>cgtttcccag atggccagat ttggaaacca gacattcttc tctataacag tgctgatgag 360<br>cgctttgacg ccacattcca cactaacgtg ttggtgaact cttctgggca ttgccagtac 420<br>ctgcctccag gcatattcaa gagttcctgc tacatcgatg tacgctggtt tccctttgat 480<br>gtgcagcact gcaaactgaa gtttgggtcc tggtcttacg gaggctggtc cttggatctg 540<br>cagatgcagg aggcagatat cagtggctat atccccaatg gagaatggga cctagtggga 600<br>atccccggca agaggagtga aaggttctat gagtgctgca aagagcccta ccccgatgtc 660<br>accttcacag tgaccatgga gcggcagatg ggttactacc tgattcagat gtatattccc 720<br>agcctgctca ttgtcatcct ctcatggatc tccttctgga tcaacatgga tgctgcacct 780<br>gctcgtgtgg gcctaggcat caccactgtg ctcaccatga ccacccagag ctccggctct 840<br>gagatcatgc ccgcaacatc cgattcggta ccattgatag cccaggccat tgacatttgg 900<br>atggcagttt gcctgctctt tgtgttctca gccctattag aatatgctgc cgttaacttt 960<br>gtgtctcggc aacataagga gctgctccga ttcaggagga agcggagaca tcacaagagc 1020<br>cccatgttga atctattcca ggaggatgaa gctggagaag gccgctttaa cttctctgcc 1080<br>tatgggatgg gcccagcctg tctacaggcc aaggatggca tctcagtcaa gggcgccaac 1140<br>aacagtaaca ccaccaaccc ccctcctgca ccatctaagt ccccagagga gatgcgaaaa 1200<br>ctcttcatcc agagggccaa gaagatcgac aaaatatccc gcattggctt ccccatggcc 1260<br>ttcctcattt tcaacatgtt ctactggatc atctacaaga ttgtccgtag agaggacgtc 1320<br>cacaaccagt ga 1332 |
| **3-27** | **Sequences** | |
| 3-27-1 | Sequence Number [ID] | 27 |
| 3-27-2 | Molecule Type | AA |
| 3-27-3 | Length | 443 |
| 3-27-4 | Features Location/ | **REGION 1..443** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..443** |

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|---|---|---|
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-27-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDEKNQ VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV RFPDGQIWKP DILLYNSADE 120<br>RFDATFHTNV LVNSSGHCQY LPPGIFKSSC YIDVRWFPFD VQHCKLKFGS WSYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFY ECCKEPYPDV TFTVTMERQM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS EIMPATSDSV PLIAQAIDIW 300<br>MAVCLLFVFS ALLEYAAVNF VSRQHKELLR FRRKRRHHKS PMLNLFQEDE AGEGRFNFSA 360<br>YGMGPACLQA KDGISVKGAN NSNTTNPPPA PSKSPEEMRK LFIQRAKKID KISRIGFPMA 420<br>FLIFNMFYWI IYKIVRREDV HNQ 443 |
| **3-28** | **Sequences** | |
| 3-28-1 | Sequence Number [ID] | 28 |
| 3-28-2 | Molecule Type | DNA |
| 3-28-3 | Length | 1332 |
| 3-28-4 | Features Location/ | **misc_feature 1..1332** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..1332** |
| | | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-28-5 | Residues | atgcgctgct cgccgggagg cgtctggctg gcgctggccg cgtcgctcct gcacgtgtcc 60<br>ctgcaaggcg agttccagag gaagctttac aaggagctgg tcaagaacta caatcccttg 120<br>gagaggcccg tggccaatga ctcgcaacca ctcaccgtct acttctccct gagcctcctg 180<br>cagatcatgg acgtggatga gaagaaccaa gttttaacca ccaacatttg gctgcaaatg 240<br>tcttggacag atcactattt acagtggaat gtgtcagaat atccaggggt gaagactgtt 300<br>cgtttcccag atggccagat ttggaaacca gacattcttc tctataacag tgctgatgag 360<br>cgctttgacg ccacattcca cactaacgtg ttggtgaact cttctgggca ttgccagtac 420<br>ctgcctccag gcatattcaa gagttcctgc tacatcgatg tacgctggtt tccctttgat 480<br>gtgcagcact gcaaactgaa gtttgggtcc tggtcttacg gaggctggtc cttggatctg 540<br>cagatgcagg aggcagatat cagtggctat atccccaatg gagaatggga cctagtggga 600<br>atccccggca agaggagtga aaggttctat gagtgctgca aagagcccta ccccgatgtc 660<br>accttcacag tgaccatgcg ccgcaggacg ggttactacc tgattcagat gtatattccc 720<br>agcctgctca ttgtcatcct ctcatggatc tccttctgga tcaacatgga tgctgcacct 780<br>gctcgtgtgg gcctaggcat caccactgtg ctcaccatga ccacccagag ctccggctct 840<br>gagatcatgc ccgcaacatc cgattcggta ccattgatag cccaggccat tgacatttgg 900<br>atggcagttt gcctgctctt tgtgttctca gccctattag aatatgctgc cgttaacttt 960<br>gtgtctcggc aacataagga gctgctccga ttcaggagga agcggagaca tcacaagagc 1020<br>cccatgttga atctattcca ggaggatgaa gctggagaag gccgctttaa cttctctgcc 1080<br>tatgggatgg gcccagcctg tctacaggcc aaggatggca tctcagtcaa gggcgccaac 1140<br>aacagtaaca ccaccaaccc ccctcctgca ccatctaagt ccccagagga gatgcgaaaa 1200<br>ctcttcatcc agagggccaa gaagatcgac aaaatatccc gcattggctt ccccatggcc 1260<br>ttcctcattt tcaacatgtt ctactggatc atctacaaga ttgtccgtag agaggacgtc 1320<br>cacaaccagt ga 1332 |
| **3-29** | **Sequences** | |
| 3-29-1 | Sequence Number [ID] | 29 |
| 3-29-2 | Molecule Type | AA |
| 3-29-3 | Length | 443 |
| 3-29-4 | Features Location/ | **REGION 1..443** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..443** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-29-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDEKNQ VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV RFPDGQIWKP DILLYNSADE 120<br>RFDATFHTNV LVNSSGHCQY LPPGIFKSSC YIDVRWFPFD VQHCKLKFGS WSYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFY ECCKEPYPDV TFTVTMRRRT GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS EIMPATSDSV PLIAQAIDIW 300<br>MAVCLLFVFS ALLEYAAVNF VSRQHKELLR FRRKRRHHKS PMLNLFQEDE AGEGRFNFSA 360<br>YGMGPACLQA KDGISVKGAN NSNTTNPPPA PSKSPEEMRK LFIQRAKKID KISRIGFPMA 420<br>FLIFNMFYWI IYKIVRREDV HNQ 443 |
| **3-30** | **Sequences** | |
| 3-30-1 | Sequence Number [ID] | 30 |
| 3-30-2 | Molecule Type | DNA |

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|---|---|---|
| 3-30-3 | Length | 1332 |
| 3-30-4 | Features Location/ | **misc_feature 1..1332** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..1332** |
| | | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-30-5 | Residues | atgcgctgct cgccgggagg cgtctggctg gcgctggccg cgtcgctcct gcacgtgtcc 60<br>ctgcaaggcg agttccagag gaagctttac aaggagctgg tcaagaacta caatcccttg 120<br>gagaggcccg tggccaatga ctcgcaacca ctcaccgtct acttctccct gagcctcctg 180<br>cagatcatgg acgtggatga gaagaaccaa gttttaacca ccaacatttg gctgcaaatg 240<br>tcttggacag atcactattt acagtggaat gtgtcagaat atccaggggt gaagactgtt 300<br>cgtttcccag atggccagat ttggaaacca gacattcttc tctataacag tgctgatgag 360<br>cgctttgacg ccacattcca cactaacgtg ttggtgaact cttctgggca ttgccagtac 420<br>ctgcctccag gcatattcaa gagttcctgc tacatcgatg tacgctggtt tccctttgat 480<br>gtgcagcact gcaaactgaa gtttgggtcc tggtcttacg gaggctggtc cttggatctg 540<br>cagatgcagg aggcagatat cagtggctat atccccaatg gagaatggga cctagtggga 600<br>atccccggca agaggagtga aaggttctat gagtgctgca aagagcccta ccccgatgtc 660<br>accttcacag tgaccatgcg ccgcaggacg ctctactacc tgattcagat gtatattccc 720<br>agcctgctca ttgtcatcct ctcatggatc tccttctgga tcaacatgga tgctgcacct 780<br>gctcgtgtgg gcctaggcat caccactgtg ctcaccatga ccacccagag ctccggctct 840<br>gagatcatgc ccgcaacatc cgattcggta ccattgatag cccaggccat tgacatttgg 900<br>atggcagttt gcctgctctt tgtgttctca gccctattag aatatgctgc cgttaacttt 960<br>gtgtctcggc aacataagga gctgctccga ttcaggagga agcggagaca tcacaagagc 1020<br>cccatgttga atctattcca ggaggatgaa gctggagaag gccgctttaa cttctctgcc 1080<br>tatgggatgg gcccagcctg tctacaggcc aaggatggca tctcagtcaa gggcgccaac 1140<br>aacagtaaca ccaccaaccc ccctcctgca ccatctaagt ccccagagga gatgcgaaaa 1200<br>ctcttcatcc agagggccaa gaagatcgac aaaatatccc gcattggctt ccccatggcc 1260<br>ttcctcattt tcaacatgtt ctactggatc atctacaaga ttgtccgtag agaggacgtc 1320<br>cacaaccagt ga 1332 |
| **3-31** | **Sequences** | |
| 3-31-1 | Sequence Number [ID] | 31 |
| 3-31-2 | Molecule Type | AA |
| 3-31-3 | Length | 443 |
| 3-31-4 | Features Location/ | **REGION 1..443** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..443** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-31-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDEKNQ VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV RFPDGQIWKP DILLYNSADE 120<br>RFDATFHTNV LVNSSGHCQY LPPGIFKSSC YIDVRWFPFD VQHCKLKFGS WSYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFY ECCKEPYPDV TFTVTMRRRT LYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS EIMPATSDSV PLIAQAIDIW 300<br>MAVCLLFVFS ALLEYAAVNF VSRQHKELLR FRRKRRHHKS PMLNLFQEDE AGEGRFNFSA 360<br>YGMGPACLQA KDGISVKGAN NSNTTNPPPA PSKSPEEMRK LFIQRAKKID KISRIGFPMA 420<br>FLIFNMFYWI IYKIVRREDV HNQ 443 |
| **3-32** | **Sequences** | |
| 3-32-1 | Sequence Number [ID] | 32 |
| 3-32-2 | Molecule Type | DNA |
| 3-32-3 | Length | 1320 |
| 3-32-4 | Features Location/ | **misc_feature 1..1320** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..1320** |
| | | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-32-5 | Residues | atgcgctgct cgccgggagg cgtctggctg gcgctggccg cgtcgctcct gcacgtgtcc 60<br>ctgcaaggcg agttccagag gaagctttac aaggagctgg tcaagaacta caatcccttg 120<br>gagaggcccg tggccaatga ctcgcaacca ctcaccgtct acttctccct gagcctcctg 180<br>cagatcatgg acgtggatga gaagaaccaa gttttaacca ccaacatttg gctgcaaatg 240<br>tcttggacag atcactattt acagtggaat gtgtcagaat atccaggggt gaagactgtt 300<br>cgtttcccag atggccagat ttggaaacca gacattcttc tctataacag tgctgatgag 360<br>cgctttgacg ccacattcca cactaacgtg ttggtgaact cttctgggca ttgccagtac 420 |

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|---|---|---|
| | | ctgcctccag gcatattcaa gagttcctgc cccatggact tgaagaattt ccccatggat 480<br>gtccagacat gcaaactgaa gtttgggtcc tggtcttacg gaggctggtc cttggatctg 540<br>cagatgcagg aggcagatat cagtggctat atccccaatg gagaatggga cctagtggga 600<br>atccccggca agaggagtga aaggttctat gagtgctgca aagagcccta ccccgatgtc 660<br>accttcacag tgaccatgcg ccgcaggatg ggttactacc tgattcagat gtatattccc 720<br>agcctgctca ttgtcatcct ctcatggatc tccttctgga tcaacatgga tgctgcacct 780<br>gctcgtgtgg gcctaggcat caccactgtg ctcaccatga ccacccagag ctccggctct 840<br>cgagcatctc tgcccaaggt gtcctatgtg aaagccattg acatttggat ggcagtttgc 900<br>ctgctctttg tgttctcagc cctattagaa tatgctgccg ttaactttgt gtctcggcaa 960<br>cataaggagc tgctccgatt caggaggaag cggagacatc acaagagccc catgttgaat 1020<br>ctattccagg aggatgaagc tggagaaggc cgctttaact tctctgccta tgggatgggc 1080<br>ccagcctgtc tacaggccaa ggatggcatc tcagtcaagg gcgccaacaa cagtaacacc 1140<br>accaaccccc ctcctgcacc atctaagtcc ccagaggaga tgcgaaaact cttcatccag 1200<br>agggccaaga agatcgacaa aatatcccgc attggcttcc ccatggcctt cctcattttc 1260<br>aacatgttct actggatcat ctacaagatt gtccgtagag aggacgtcca caaccagtga 1320 |
| **3-33** | **Sequences** | |
| 3-33-1 | Sequence Number [ID] | 33 |
| 3-33-2 | Molecule Type | AA |
| 3-33-3 | Length | 439 |
| 3-33-4 | Features Location/ | **REGION 1..439** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..439** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-33-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDEKNQ VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV RFPDGQIWKP DILLYNSADE 120<br>RFDATFHTNV LVNSSGHCQY LPPGIFKSSC PMDLKNFPMD VQTCKLKFGS WSYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFY ECCKEPYPDV TFTVTMRRRM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS RASLPKVSYV KAIDIWMAVC 300<br>LLFVFSALLE YAAVNFVSRQ HKELLRFRRK RRHHKSPMLN LFQEDEAGEG RFNFSAYGMG 360<br>PACLQAKDGI SVKGANNSNT TNPPPAPSKS PEEMRKLFIQ RAKKIDKISR IGFPMAFLIF 420<br>NMFYWIIYKI VRREDVHNQ 439 |
| **3-34** | **Sequences** | |
| 3-34-1 | Sequence Number [ID] | 34 |
| 3-34-2 | Molecule Type | DNA |
| 3-34-3 | Length | 1320 |
| 3-34-4 | Features Location/ | **misc_feature 1..1320** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..1320** |
| | | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-34-5 | Residues | atgcgctgct cgccgggagg cgtctggctg gcgctggccg cgtcgctcct gcacgtgtcc 60<br>ctgcaaggcg agttccagag gaagctttac aaggagctgg tcaagaacta caatcccttg 120<br>gagaggcccg tggccaatga ctcgcaacca ctcaccgtct acttctccct gagcctcctg 180<br>cagatcatgg acgtggatga gaagaaccaa gttttaacca ccaacatttg gctgcaaatg 240<br>tcttggacag atcactattt acagtggaat gtgtcagaat atccaggggt gaagactgtt 300<br>cgtttcccag atggccagat ttggaaacca gacattcttc tctataacag tgctgatgag 360<br>cgctttgacg ccacattcca cactaacgtg ttggtgaact cttctgggca ttgccagtac 420<br>ctgcctccag gcatattcaa gagttcctgc cccatggact tgaagaattt tcccctttgat 480<br>gtgcagcact gcaaactgaa gtttgggtcc tggtcttacg gaggctggtc cttggatctg 540<br>cagatgcagg aggcagatat cagtggctat atccccaatg gagaatggga cctagtggga 600<br>atccccggca agaggagtga aaggttctat gagtgctgca aagagcccta ccccgatgtc 660<br>accttcacag tgaccatgcg ccgcaggatg ggttactacc tgattcagat gtatattccc 720<br>agcctgctca ttgtcatcct ctcatggatc tccttctgga tcaacatgga tgctgcacct 780<br>gctcgtgtgg gcctaggcat caccactgtg ctcaccatga ccacccagag ctccggctct 840<br>cgagcatctc tgcccaaggt gtcctatgtg aaagccattg acatttggat ggcagtttgc 900<br>ctgctctttg tgttctcagc cctattagaa tatgctgccg ttaactttgt gtctcggcaa 960<br>cataaggagc tgctccgatt caggaggaag cggagacatc acaagagccc catgttgaat 1020<br>ctattccagg aggatgaagc tggagaaggc cgctttaact tctctgccta tgggatgggc 1080<br>ccagcctgtc tacaggccaa ggatggcatc tcagtcaagg gcgccaacaa cagtaacacc 1140<br>accaaccccc ctcctgcacc atctaagtcc ccagaggaga tgcgaaaact cttcatccag 1200<br>agggccaaga agatcgacaa aatatcccgc attggcttcc ccatggcctt cctcattttc 1260<br>aacatgttct actggatcat ctacaagatt gtccgtagag aggacgtcca caaccagtga 1320 |

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|---|---|---|
| **3-35** | **Sequences** | |
| 3-35-1 | Sequence Number [ID] | 35 |
| 3-35-2 | Molecule Type | AA |
| 3-35-3 | Length | 439 |
| 3-35-4 | Features Location/ | **REGION 1..439** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..439** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-35-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDEKNQ VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV RFPDGQIWKP DILLYNSADE 120<br>RFDATFHTNV LVNSSGHCQY LPPGIFKSSC PMDLKNFPFD VQHCKLKFGS WSYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFY ECCKEPYPDV TFTVTMRRRM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS RASLPKVSYV KAIDIWMAVC 300<br>LLFVFSALLE YAAVNFVSRQ HKELLRFRRK RRHHKSPMLN LFQEDEAGEG RFNFSAYGMG 360<br>PACLQAKDGI SVKGANNSNT TNPPPAPSKS PEEMRKLFIQ RAKKIDKISR IGFPMAFLIF 420<br>NMFYWIIYKI VRREDVHNQ 439 |
| **3-36** | **Sequences** | |
| 3-36-1 | Sequence Number [ID] | 36 |
| 3-36-2 | Molecule Type | DNA |
| 3-36-3 | Length | 1320 |
| 3-36-4 | Features Location/ | **misc_feature 1..1320** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..1320** |
| | | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-36-5 | Residues | atgcgctgct cgccgggagg cgtctggctg gcgctggccg cgtcgctcct gcacgtgtcc 60<br>ctgcaaggcg agttccagag gaagctttac aaggagctgg tcaagaacta caatcccttg 120<br>gagaggcccg tggccaatga ctcgcaacca ctcaccgtct acttctccct gagcctcctg 180<br>cagatcatgg acgtggatga gacaaccatg gttttaacca ccaacatttg gctgcaaatg 240<br>tcttggacag atcactattt acagtggaat gtgtcagaat atccaggggt gaagactgtt 300<br>cgtttcccag atggccagat ttggaaacca gacattcttc tctataacag tgctgatgag 360<br>cgctttgacg ccacattcca cactaacgtg ttggtgaact cttctgggca ttgccagtac 420<br>ctgcctccag gcatattcaa gagttcctgc tacatcgatg tacgctggtt tccctttgat 480<br>gtgcagcact gcaaactgaa gtttgggtcc tggtcttacg gaggctggtc cttggatctg 540<br>cagatgcagg aggcagatat cagtggctat atccccaatg gagaatggga cctagtggga 600<br>atccccggca agaggagtga aaggttctat gagtgctgca aagagcccta ccccgatgtc 660<br>accttcacag tgaccatgcg ccgcaggatg ggttactacc tgattcagat gtatattccc 720<br>agcctgctca ttgtcatcct ctcatggatc tccttctgga tcaacatgga tgctgcacct 780<br>gctcgtgtgg gcctaggcat caccactgtg ctcaccatga ccacccagag ctccggctct 840<br>cgagcatctc tgcccaaggt gtcctatgtg aaagccattg acatttggat ggcagtttgc 900<br>ctgctctttg tgttctcagc cctattagaa tatgctgccg ttaactttgt gtctcggcaa 960<br>cataaggagc tgctccgatt caggaggaag cggagacatc acaagagccc catgttgaat 1020<br>ctattccagg aggatgaagc tggagaaggc cgctttaact tctctgccta tgggatgggc 1080<br>ccagcctgtc tacaggccaa ggatggcatc tcagtcaagg gcgccaacaa cagtaacacc 1140<br>accaaccccc ctcctgcacc atctaagtcc ccagaggaga tgcgaaaact cttcatccag 1200<br>agggccaaga agatcgacaa aatatcccgc attggcttcc ccatggcctt cctcattttc 1260<br>aacatgttct actggatcat ctacaagatt gtccgtagag aggacgtcca caaccagtga 1320 |
| **3-37** | **Sequences** | |
| 3-37-1 | Sequence Number [ID] | 37 |
| 3-37-2 | Molecule Type | AA |
| 3-37-3 | Length | 439 |
| 3-37-4 | Features Location/ | **REGION 1..439** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..439** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-37-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDETTM VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV RFPDGQIWKP DILLYNSADE 120<br>RFDATFHTNV LVNSSGHCQY LPPGIFKSSC YIDVRWFPFD VQHCKLKFGS WSYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFY ECCKEPYPDV TFTVTMRRRM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS RASLPKVSYV KAIDIWMAVC 300 |

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|---|---|---|
| | | LLFVFSALLE YAAVNFVSRQ HKELLRFRRK RRHHKSPMLN LFQEDEAGEG RFNFSAYGMG 360<br>PACLQAKDGI SVKGANNSNT TNPPPAPSKS PEEMRKLFIQ RAKKIDKISR IGFPMAFLIF 420<br>NMFYWIIYKI VRREDVHNQ 439 |
| **3-38** | **Sequences** | |
| 3-38-1 | Sequence Number [ID] | 38 |
| 3-38-2 | Molecule Type | DNA |
| 3-38-3 | Length | 1320 |
| 3-38-4 | Features Location/ | **misc_feature 1..1320** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..1320** |
| | | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-38-5 | Residues | atgcgctgct cgccgggagg cgtctggctg gcgctggccg cgtcgctcct gcacgtgtcc 60<br>ctgcaaggcg agttccagag gaagctttac aaggagctgg tcaagaacta caatcccttg 120<br>gagaggcccg tggccaatga ctcgcaacca ctcaccgtct acttctccct gagcctcctg 180<br>cagatcatgg acattgctga gacaaccatg gacttaacca ccaacatttg gctgcaaatg 240<br>tcttggacag atcactattt acagtggaat gtgtcagaat atccaggggt gaagactgtt 300<br>cgtttcccag atggccagat ttggaaacca gacattcttc tctataacag tgctgatgag 360<br>cgctttgacg ccacattcca cactaacgtg ttggtgaact cttctgggca ttgccagtac 420<br>ctgcctccag gcatattcaa gagttcctgc cccatggact tgaagaattt ccccatggat 480<br>gtccagacat gcaaactgaa gtttgggtcc tggtcttacg gaggctggtc cttggatctg 540<br>cagatgcagg aggcagatat cagtggctat atccccaatg gagaatggga cctagtggga 600<br>atccccggca agaggagtga aaggttctat gagtgctgca aagagcccta ccccgatgtc 660<br>accttcacag tgaccatgcg ccgcaggatg ggttactacc tgattcagat gtatattccc 720<br>agcctgctca ttgtcatcct ctcatggatc tccttctgga tcaacatgga tgctgcacct 780<br>gctcgtgtgg gcctaggcat caccactgtg ctcaccatga ccacccagag ctccggctct 840<br>cgagcatctc tgcccaaggt gtcctatgtg aaagccattg acatttggat ggcagtttgc 900<br>ctgctctttg tgttctcagc cctattagaa tatgctgccg ttaactttgt gtctcggcaa 960<br>cataaggagc tgctccgatt caggaggaag cggagacatc acaagagccc catgttgaat 1020<br>ctattccagg aggatgaagc tggagaaggc cgctttaact tctctgccta tgggatgggc 1080<br>ccagcctgtc tacaggccaa ggatggcatc tcagtcaagg gcgccaacaa cagtaacacc 1140<br>accaaccccc ctcctgcacc atctaagtcc ccagaggaga tgcgaaaact cttcatccag 1200<br>agggccaaga agatcgacaa aatatcccgc attggcttcc ccatggcctt cctcatttc 1260<br>aacatgttct actggatcat ctacaagatt gtccgtagag aggacgtcca caaccagtga 1320 |
| **3-39** | **Sequences** | |
| 3-39-1 | Sequence Number [ID] | 39 |
| 3-39-2 | Molecule Type | AA |
| 3-39-3 | Length | 439 |
| 3-39-4 | Features Location/ | **REGION 1..439** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..439** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-39-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDIAETTM DLTTNIWLQM SWTDHYLQWN VSEYPGVKTV RFPDGQIWKP DILLYNSADE 120<br>RFDATFHTNV LVNSSGHCQY LPPGIFKSSC PMDLKNFPMD VQTCKLKFGS WSYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFY ECCKEPYPDV TFTVTMRRRM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS RASLPKVSYV KAIDIWMAVC 300<br>LLFVFSALLE YAAVNFVSRQ HKELLRFRRK RRHHKSPMLN LFQEDEAGEG RFNFSAYGMG 360<br>PACLQAKDGI SVKGANNSNT TNPPPAPSKS PEEMRKLFIQ RAKKIDKISR IGFPMAFLIF 420<br>NMFYWIIYKI VRREDVHNQ 439 |
| **3-40** | **Sequences** | |
| 3-40-1 | Sequence Number [ID] | 40 |
| 3-40-2 | Molecule Type | DNA |
| 3-40-3 | Length | 1320 |
| 3-40-4 | Features Location/ | **misc_feature 1..1320** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..1320** |
| | | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-40-5 | Residues | atgcgctgct cgccgggagg cgtctggctg gcgctggccg cgtcgctcct gcacgtgtcc 60<br>ctgcaaggcg agttccagag gaagctttac aaggagctgg tcaagaacta caatcccttg 120 |

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|---|---|---|
| | | gagaggcccg tggccaatga ctcgcaacca ctcaccgtct acttctccct gagcctcctg 180<br>cagatcatgg acgtggatga gacaaccatg gttttaacca ccaacatttg gctgcaaatg 240<br>tcttggacag atcactattt acagtggaat gtgtcagaat atccaggggt gaagactgtt 300<br>cgtttcccag atggccagat ttggaaacca gacattcttc tctataacag tgctgatgag 360<br>cgctttgacg ccacattcca cactaacgtg ttggtgaact cttctgggca ttgccagtac 420<br>ctgcctccag gcatattcaa gagttcctgc cccatggact tgaagaattt ccccatggat 480<br>gtccagacat gcaaactgaa gtttgggtcc tggtcttacg gaggctggtc cttggatctg 540<br>cagatgcagg aggcagatat cagtggctat atccccaatg gagaatggga cctagtggga 600<br>atccccggca agaggagtga aaggttctat gagtgctgca aagagcccta ccccgatgtc 660<br>accttcacag tgaccatgcg ccgcaggatg ggttactacc tgattcagat gtatattccc 720<br>agcctgctca ttgtcatcct ctcatggatc tccttctgga tcaacatgga tgctgcacct 780<br>gctcgtgtgg gcctaggcat caccactgtg ctcaccatga ccacccagag ctccggctct 840<br>cgagcatctc tgcccaaggt gtcctatgtg aaagccattg acatttggat ggcagtttgc 900<br>ctgctctttg tgttctcagc cctattagaa tatgctgccg ttaactttgt gtctcggcaa 960<br>cataaggagc tgctccgatt caggaggaag cggagacatc acaagagccc catgttgaat 1020<br>ctattccagg aggatgaagc tggagaaggc cgctttaact tctctgccta tgggatgggc 1080<br>ccagcctgtc tacaggccaa ggatggcatc tcagtcaagg gcgccaacaa cagtaacacc 1140<br>accaaccccc ctcctgcacc atctaagtcc ccagaggaga tgcgaaaact cttcatccag 1200<br>agggccaaga agatcgacaa aatatcccgc attggcttcc ccatggcctt cctcattttc 1260<br>aacatgttct actggatcat ctacaagatt gtccgtagag aggacgtcca caaccagtga 1320 |
| **3-41** | **Sequences** | |
| 3-41-1 | Sequence Number [ID] | 41 |
| 3-41-2 | Molecule Type | AA |
| 3-41-3 | Length | 439 |
| 3-41-4 | Features Location/ | **REGION 1..439** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..439** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-41-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDETTM VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV RFPDGQIWKP DILLYNSADE 120<br>RFDATFHTNV LVNSSGHCQY LPPGIFKSSC PMDLKNFPMD VQTCKLKFGS WSYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFY ECCKEPYPDV TFTVTMRRRM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS RASLPKVSYV KAIDIWMAVC 300<br>LLFVFSALLE YAAVNFVSRQ HKELLRFRRK RRHHKSPMLN LFQEDEAGEG RFNFSAYGMG 360<br>PACLQAKDGI SVKGANNSNT TNPPPAPSKS PEEMRKLFIQ RAKKIDKISR IGFPMAFLIF 420<br>NMFYWIIYKI VRREDVHNQ 439 |
| **3-42** | **Sequences** | |
| 3-42-1 | Sequence Number [ID] | 42 |
| 3-42-2 | Molecule Type | DNA |
| 3-42-3 | Length | 1317 |
| 3-42-4 | Features Location/ | **misc_feature 1..1317** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..1317** |
| | | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-42-5 | Residues | atgcgctgct cgccgggagg cgtctggctg gcgctggccg cgtcgctcct gcacgtgtcc 60<br>ctgcaaggcg agttccagag gaagctttac aaggagctgg tcaagaacta caatcccttg 120<br>gagaggcccg tggccaatga ctcgcaacca ctcaccgtct acttctccct gagcctcctg 180<br>cagatcatgg acgtggatga gacaaccatg gttttaacca ccaacatttg gctgcaaatg 240<br>tcttggacag atcactattt acagtggaat gtgtcagaat atccaggggt gaagactgtt 300<br>cgtttcccag atggccagat ttggaaacca gacattcttc tctataacag tgctgatgag 360<br>cgctttgacg ccacattcca cactaacgtg ttggtgaact cttctgggca ttgccagtac 420<br>ctgcctccag gcatattcaa gagttcctgc cccatggact tgaagaattt ccccatggat 480<br>gtccagacat gcaaactgaa gtttgggtcc tggtcttacg gaggctggtc cttggatctg 540<br>cagatgcagg aggcagatat cagtggctat atccccaatg gagaatggga cctagtggga 600<br>atccccggca agaggagtga aaggttctat gagtgctgca aagagcccta ccccgatgtc 660<br>accttcacag tgaccatgga gcggcagatg ggttactacc tgattcagat gtatattccc 720<br>agcctgctca ttgtcatcct ctcatggatc tccttctgga tcaacatgga tgctgcacct 780<br>gctcgtgtgg gcctaggcat caccactgtg ctcaccatga ccacccagag ctccggctct 840<br>cgagcatctc tgcccaaggt gtcctatgtg aaagccattg acatttggat ggcagtttgc 900<br>ctgctctttg tgttctcagc cctattagaa tatgctgccg ttaactttgt gtctcggcaa 960<br>cataaggagc tgctccgatt caggaggaag cggagacatc acaagagccc catgttgaat 1020 |

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|---|---|---|
| | | ctattccagg aggatgaagc tggagaaggc cgctttaact tctctgccta tgggatgggc 1080<br>ccagcctgtc tacaggccaa ggatggcatc tcagtcaagg gcgccaacaa cagtaacacc 1140<br>accaaccccc ctcctgcacc atctaagtcc ccagaggaga tgcgaaaact cttcatccag 1200<br>agggccaaga agatcgacaa aatatcccgc attggcttcc ccatggcctt cctcattttc 1260<br>aacatgttct actggatcat ctacaagatt gtccgtagag aggacgtcca caaccag 1317 |
| **3-43** | **Sequences** | |
| 3-43-1 | Sequence Number [ID] | 43 |
| 3-43-2 | Molecule Type | AA |
| 3-43-3 | Length | 439 |
| 3-43-4 | Features Location/ | **REGION 1..439** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..439** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-43-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDETTM VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV RFPDGQIWKP DILLYNSADE 120<br>RFDATFHTNV LVNSSGHCQY LPPGIFKSSC PMDLKNFPMD VQTCKLKFGS WSYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFY ECCKEPYPDV TFTVTMERQM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS RASLPKVSYV KAIDIWMAVC 300<br>LLFVFSALLE YAAVNFVSRQ HKELLRFRRK RRHHKSPMLN LFQEDEAGEG RFNFSAYGMG 360<br>PACLQAKDGI SVKGANNSNT TNPPPAPSKS PEEMRKLFIQ RAKKIDKISR IGFPMAFLIF 420<br>NMFYWIIYKI VRREDVHNQ 439 |
| **3-44** | **Sequences** | |
| 3-44-1 | Sequence Number [ID] | 44 |
| 3-44-2 | Molecule Type | DNA |
| 3-44-3 | Length | 1317 |
| 3-44-4 | Features Location/ | **misc_feature 1..1317** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..1317** |
| | | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-44-5 | Residues | atgcgctgct cgccgggagg cgtctggctg gcgctggccg cgtcgctcct gcacgtgtcc 60<br>ctgcaaggcg agttccagag gaagctttac aaggagctgg tcaagaacta caatcccttg 120<br>gagaggcccg tggccaatga ctcgcaacca ctcaccgtct acttctccct gagcctcctg 180<br>cagatcatgg acattgctga gacaaccatg gacttaacca ccaacatttg gctgcaaatg 240<br>tcttggacag atcactattt acagtggaat gtgtcagaat atccaggggt gaagactgtt 300<br>cgtttcccag atggccagat ttggaaacca gacattcttc tctataacag tgctgatgag 360<br>cgctttgacg ccacattcca cactaacgtg ttggtgaact cttctgggca ttgccagtac 420<br>ctgcctccag gcatattcaa gagttcctgc cccatggact tgaagaattt ccccatggat 480<br>gtccagacat gcaaactgaa gtttgggtcc tggtcttacg gaggctggtc cttggatctg 540<br>cagatgcagg aggcagatat cagtggctat atccccaatg gagaatggga cctagtggga 600<br>atccccggca agaggagtga aaggttctat gagtgctgca aagagcccta ccccgatgtc 660<br>accttcacag tgaccatgga gcggcagatg ggttactacc tgattcagat gtatattccc 720<br>agcctgctca ttgtcatcct ctcatggatc tccttctgga tcaacatgga tgctgcacct 780<br>gctcgtgtgg gcctaggcat caccactgtg ctcaccatga ccacccagag ctccggctct 840<br>cgagcatctc tgcccaaggt gtcctatgtg aaagccattg acatttggat ggcagtttgc 900<br>ctgctctttg tgttctcagc cctattagaa tatgctgccg ttaactttgt gtctcggcaa 960<br>cataaggagc tgctccgatt caggaggaag cggagacatc acaagagccc catgttgaat 1020<br>ctattccagg aggatgaagc tggagaaggc cgctttaact tctctgccta tgggatgggc 1080<br>ccagcctgtc tacaggccaa ggatggcatc tcagtcaagg gcgccaacaa cagtaacacc 1140<br>accaaccccc ctcctgcacc atctaagtcc ccagaggaga tgcgaaaact cttcatccag 1200<br>agggccaaga agatcgacaa aatatcccgc attggcttcc ccatggcctt cctcattttc 1260<br>aacatgttct actggatcat ctacaagatt gtccgtagag aggacgtcca caaccag 1317 |
| **3-45** | **Sequences** | |
| 3-45-1 | Sequence Number [ID] | 45 |
| 3-45-2 | Molecule Type | AA |
| 3-45-3 | Length | 439 |
| 3-45-4 | Features Location/ | **REGION 1..439** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..439** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |

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|---|---|---|
| 3-45-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDIAETTM DLTTNIWLQM SWTDHYLQWN VSEYPGVKTV RFPDGQIWKP DILLYNSADE 120<br>RFDATFHTNV LVNSSGHCQY LPPGIFKSSC PMDLKNFPMD VQTCKLKFGS WSYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFY ECCKEPYPDV TFTVTMERQM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS RASLPKVSYV KAIDIWMAVC 300<br>LLFVFSALLE YAAVNFVSRQ HKELLRFRRK RRHHKSPMLN LFQEDEAGEG RFNFSAYGMG 360<br>PACLQAKDGI SVKGANNSNT TNPPPAPSKS PEEMRKLFIQ RAKKIDKISR IGFPMAFLIF 420<br>NMFYWIIYKI VRREDVHNQ 439 |
| **3-46** | **Sequences** | |
| 3-46-1 | Sequence Number [ID] | 46 |
| 3-46-2 | Molecule Type | DNA |
| 3-46-3 | Length | 1329 |
| 3-46-4 | Features Location/ | **misc_feature 1..1329** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..1329** |
| | | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-46-5 | Residues | atgcgctgct cgccgggagg cgtctggctg gcgctggccg cgtcgctcct gcacgtgtcc 60<br>ctgcaaggcg agttccagag gaagctttac aaggagctgg tcaagaacta caatcccttg 120<br>gagaggcccg tggccaatga ctcgcaacca ctcaccgtct acttctccct gagcctcctg 180<br>cagatcatgg acgtggatga gacaaccatg gttttaacca ccaacatttg gctgcaaatg 240<br>tcttggacag atcactattt acagtggaat gtgtcagaat atccaggggt gaagactgtt 300<br>cgtttcccag atggccagat ttggaaacca gacattcttc tctataacag tgctgatgag 360<br>cgctttgacg ccacattcca cactaacgtg ttggtgaact cttctgggca ttgccagtac 420<br>ctgcctccag gcatattcaa gagttcctgc tacatcgatg tacgctggtt tccctttgat 480<br>gtgcagcact gcaaactgaa gtttgggtcc tggtcttacg gaggctggtc cttggatctg 540<br>cagatgcagg aggcagatat cagtggctat atccccaatg gagaatggga cctagtggga 600<br>atccccggca agaggagtga aaggttctat gagtgctgca aagagcccta ccccgatgtc 660<br>accttcacag tgaccatgcg ccgcaggatg ggttactacc tgattcagat gtatattccc 720<br>agcctgctca ttgtcatcct ctcatggatc tccttctgga tcaacatgga tgctgcacct 780<br>gctcgtgtgg gcctaggcat caccactgtg ctcaccatga ccacccagag ctccggctct 840<br>gagatcatgc ccgcaacatc cgattcggta ccattgatag cccaggccat tgacatttgg 900<br>atggcagttt gcctgctctt tgtgttctca gccctattag aatatgctgc cgttaacttt 960<br>gtgtctcggc aacataagga gctgctccga ttcaggagga agcggagaca tcacaagagc 1020<br>cccatgttga atctattcca ggaggatgaa gctggagaag gccgctttaa cttctctgcc 1080<br>tatgggatgg gcccagcctg tctacaggcc aaggatggca tctcagtcaa gggcgccaac 1140<br>aacagtaaca ccaccaaccc ccctcctgca ccatctaagt ccccagagga gatgcgaaaa 1200<br>ctcttcatcc agagggccaa gaagatcgac aaaatatccc gcattggctt ccccatggcc 1260<br>ttcctcattt tcaacatgtt ctactggatc atctacaaga ttgtccgtag agaggacgtc 1320<br>cacaaccag 1329 |
| **3-47** | **Sequences** | |
| 3-47-1 | Sequence Number [ID] | 47 |
| 3-47-2 | Molecule Type | AA |
| 3-47-3 | Length | 443 |
| 3-47-4 | Features Location/ | **REGION 1..443** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..443** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-47-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDETTM VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV RFPDGQIWKP DILLYNSADE 120<br>RFDATFHTNV LVNSSGHCQY LPPGIFKSSC YIDVRWFPFD VQHCKLKFGS WSYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFY ECCKEPYPDV TFTVTMRRRM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS EIMPATSDSV PLIAQAIDIW 300<br>MAVCLLFVFS ALLEYAAVNF VSRQHKELLR FRRKRRHHKS PMLNLFQEDE AGEGRFNFSA 360<br>YGMGPACLQA KDGISVKGAN NSNTTNPPPA PSKSPEEMRK LFIQRAKKID KISRIGFPMA 420<br>FLIFNMFYWI IYKIVRREDV HNQ 443 |
| **3-48** | **Sequences** | |
| 3-48-1 | Sequence Number [ID] | 48 |
| 3-48-2 | Molecule Type | DNA |
| 3-48-3 | Length | 1332 |
| 3-48-4 | Features Location/ | **misc_feature 1..1332** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |

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|---|---|---|
| | | **source 1..1332**<br>mol_type=other DNA<br>organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-48-5 | Residues | atgcgctgct cgccgggagg cgtctggctg gcgctggccg cgtcgctcct gcacgtgtcc 60<br>ctgcaaggcg agttccagag gaagctttac aaggagctgg tcaagaacta caatcccttg 120<br>gagaggcccg tggccaatga ctcgcaacca ctcaccgtct acttctccct gagcctcctg 180<br>cagatcatgg acgtggatga gaagaaccaa gttttaacca ccaacatttg gctgcaaatg 240<br>tcttggacag atcactattt acagtggaat gtgtcagaat atccaggggt gaagactgtt 300<br>cgtttcccag atggccagat ttggaaacca gacattcttc tctataacag tgctgatgag 360<br>cgctttgacg ccacattcca cactaacgtg ttggtgaact cttctgggca ttgccagtac 420<br>ctgcctccag gcatattcaa gagttcctgc cccatggact tgaagaattt ccccatggat 480<br>gtccagacat gcaaactgaa gtttgggtcc tggtcttacg gaggctggtc cttggatctg 540<br>cagatgcagg aggcagatat cagtggctat atccccaatg gagaatggga cctagtggga 600<br>atccccggca agaggagtga aaggttctat gagtgctgca aagagcccta ccccgatgtc 660<br>accttcacag tgaccatgcg ccgcaggatg ggttactacc tgattcagat gtatattccc 720<br>agcctgctca ttgtcatcct ctcatggatc tccttctgga tcaacatgga tgctgcacct 780<br>gctcgtgtgg gcctaggcat caccactgtg ctcaccatga ccacccagag ctccggctct 840<br>gagatcatgc ccgcaacatc cgattcggta ccattgatag cccaggccat tgacatttgg 900<br>atggcagttt gcctgctctt tgtgttctca gccctattag aatatgctgc cgttaacttt 960<br>gtgtctcggc aacataagga gctgctccga ttcaggagga agcggagaca tcacaagagc 1020<br>cccatgttga atctattcca ggaggatgaa gctggagaag gccgctttaa cttctctgcc 1080<br>tatgggatgg gcccagcctg tctacaggcc aaggatggca tctcagtcaa gggcgccaac 1140<br>aacagtaaca ccaccaaccc ccctcctgca ccatctaagt ccccagagga gatgcgaaaa 1200<br>ctcttcatcc agagggccaa gaagatcgac aaaatatccc gcattggctt ccccatggcc 1260<br>ttcctcattt tcaacatgtt ctactggatc atctacaaga ttgtccgtag agaggacgtc 1320<br>cacaaccagt ga 1332 |
| **3-49** | **Sequences** | |
| 3-49-1 | Sequence Number [ID] | 49 |
| 3-49-2 | Molecule Type | AA |
| 3-49-3 | Length | 443 |
| 3-49-4 | Features Location/ Qualifiers | **REGION 1..443**<br>note=synthetic sequence of chimeric LGIC<br>**source 1..443**<br>mol_type=protein<br>organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-49-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDEKNQ VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV RFPDGQIWKP DILLYNSADE 120<br>RFDATFHTNV LVNSSGHCQY LPPGIFKSSC PMDLKNFPMD VQTCKLKFGS WSYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFY ECCKEPYPDV TFTVTMRRRM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS EIMPATSDSV PLIAQAIDIW 300<br>MAVCLLFVFS ALLEYAAVNF VSRQHKELLR FRRKRRHHKS PMLNLFQEDE AGEGRFNFSA 360<br>YGMGPACLQA KDGISVKGAN NSNTTNPPPA PSKSPEEMRK LFIQRAKKID KISRIGFPMA 420<br>FLIFNMFYWI IYKIVRREDV HNQ 443 |
| **3-50** | **Sequences** | |
| 3-50-1 | Sequence Number [ID] | 50 |
| 3-50-2 | Molecule Type | AA |
| 3-50-3 | Length | 451 |
| 3-50-4 | Features Location/ Qualifiers | **REGION 1..451**<br>note=synthetic sequence of chimeric LGIC<br>**source 1..451**<br>mol_type=protein<br>organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-50-5 | Residues | MLLWVQQALL ALLLPTLLAQ GEARRSRNTT RPALLRLSDY LLTNYRKGVR PVRDWRKPTT 60<br>VSIDVIVYAI LNVDEKNQVL TTYIWYRQYW TDEFLQWNPE DFDNITKLSI PTDSIWVPDI 120<br>LINEFVDVGK SPNIPYVYIR HQGEVQNYKP LQVVTACSLD IYNFPFDVQN CSLTFTSWLH 180<br>TIQDINISLW RLPEKVKSDR SVFMNQGEWE LLGVLPYFRE FSMESSNYYA EMKFYVVIRR 240<br>RMGYYLIQMY IPSLLIVILS WISFWINMDA APARVGLGIT TVLTMTTQSS GSRASLPKVS 300<br>YVKAIDIWMA VCLLFVFSAL LEYAAVNFVS RQHKELLRFR RKRRHHKSPM LNLFQEDEAG 360<br>EGRFNFSAYG MGPACLQAKD GISVKGANNS NTTNPPPAPS KSPEEMRKLF IQRAKKIDKI 420<br>SRIGFPMAFL IFNMFYWIIY KIVRREDVHN Q 451 |
| **3-51** | **Sequences** | |
| 3-51-1 | Sequence Number [ID] | 51 |

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|---|---|---|
| 3-51-2 | Molecule Type | AA |
| 3-51-3 | Length | 451 |
| 3-51-4 | Features Location/ | **REGION 1..451** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..451** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-51-5 | Residues | MLLWVQQALL ALLLPTLLAQ GEARRSRNTT RPALLRLSDY LLTNYRKGVR PVRDWRKPTT 60<br>VSIDVIVYAI LNVDEKNQVL TTYIWYRQYW TDEFLQWNPE DFDNITKLSI PTDSIWVPDI 120<br>LINEFVDVGK SPNIPYVYIR HQGEVQNYKP LQVVTACSLD IYNFPFDVQN CSLTFTSWLH 180<br>TIQDINISLW RLPEKVKSDR SVFMNQGEWE LLGVLPYFRE FSMESSNYYA EMKFYVHLER 240<br>QMGYYLIQMY IPSLLIVILS WISFWINMDA APARVGLGIT TVLTMTTQSS GSRASLPKVS 300<br>YVKAIDIWMA VCLLFVFSAL LEYAAVNFVS RQHKELLRFR RKRRHHKSPM LNLFQEDEAG 360<br>EGRFNFSAYG MGPACLQAKD GISVKGANNS NTTNPPPAPS KSPEEMRKLF IQRAKKIDKI 420<br>SRIGFPMAFL IFNMFYWIIY KIVRREDVHN Q 451 |
| **3-52** | **Sequences** | |
| 3-52-1 | Sequence Number [ID] | 52 |
| 3-52-2 | Molecule Type | AA |
| 3-52-3 | Length | 455 |
| 3-52-4 | Features Location/ | **REGION 1..455** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..455** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-52-5 | Residues | MWGLAGGRLF GIFSAPVLVA VVCCAQSVND PGNMSFVKET VDKLLKGYDI RLRPDFGGPP 60<br>VCVGMNIDIA SIDMVSEVNM DYTLTMYFQQ YWRDKRLAYS GIPLNLTLDN RVADQLWVPD 120<br>TYFLNDKKSF VHGVTVKNRM IRLHPDGTVL YGLRITTTAA CMMDLRRYPL DEQNCTLEIE 180<br>SYGYTTDDIE FYWRGGDKAV TGVERIELPQ FSIVEHRLVS RNVVFATGAY PRLSLSFRLK 240<br>RNIGYMGYYL IQMYIPSLLI VILSWISFWI NMDAAPARVG LGITTVLTMT TQSSGSRASL 300<br>PKVSYVKAID IWMAVCLLFV FSALLEYAAV NFVSRQHKEL LRFRRKRRHH KSPMLNLFQE 360<br>DEAGEGRFNF SAYGMGPACL QAKDGISVKG ANNSNTTNPP PAPSKSPEEM RKLFIQRAKK 420<br>IDKISRIGFP MAFLIFNMFY WIIYKIVRRE DVHNQ 455 |
| **3-53** | **Sequences** | |
| 3-53-1 | Sequence Number [ID] | 53 |
| 3-53-2 | Molecule Type | AA |
| 3-53-3 | Length | 31 |
| 3-53-4 | Features Location/ | **REGION 1..31** |
| | Qualifiers | note=C-terminal amino acids of GABAR1 |
| | | **source 1..31** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-53-5 | Residues | IDRLSRIAFP LLFGIFNLVY WATYLNREPQ L 31 |
| **3-54** | **Sequences** | |
| 3-54-1 | Sequence Number [ID] | 54 |
| 3-54-2 | Molecule Type | AA |
| 3-54-3 | Length | 22 |
| 3-54-4 | Features Location/ | **REGION 1..22** |
| | Qualifiers | note=Amino acid residues 260-281 of human CHRNA7 |
| | | **source 1..22** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-54-5 | Residues | EKISLGITVL LSLTVFMLLV AE 22 |
| **3-55** | **Sequences** | |
| 3-55-1 | Sequence Number [ID] | 55 |
| 3-55-2 | Molecule Type | AA |
| 3-55-3 | Length | 23 |
| 3-55-4 | Features Location/ | **REGION 1..23** |
| | Qualifiers | note=Synthetic polypeptide sequence |
| | | **source 1..23** |
| | | mol_type=protein |
| | | organism=synthetic construct |

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| | NonEnglishQualifier Value | |
| 3-55-5 | Residues | PAKIGLGITV LLSLTTFMSG VAN 23 |
| **3-56** | **Sequences** | |
| 3-56-1 | Sequence Number [ID] | 56 |
| 3-56-2 | Molecule Type | DNA |
| 3-56-3 | Length | 1780 |
| 3-56-4 | Features Location/ | **source 1..1780** |
| | Qualifiers | mol_type=other DNA |
| | | organism=Homo sapiens |
| | NonEnglishQualifier Value | |
| 3-56-5 | Residues | atgttgtcaa gtgtaatggc tcccctgtgg gcctgcatcc tggtggctgc aggaattcta 60<br>gccacagata cacatcatcc ccaggattct gctctgtatc atctcagcaa gcagctatta 120<br>cagaaatatc ataaagaagt gagacctgtt tacaactgga ccaaggccac cacagtctac 180<br>ctggacctgt tcgtccatgc tatattggat gtggatgcag agaatcaaat attaaagaca 240<br>agtgtatggt accaagaggt ctggaatgat gaatttttat cctggaactc cagcatgttt 300<br>gatgagatta gagagatctc cctacctcta agtgccatct gggcccccga tatcatcatc 360<br>aatgagtttg tggacattga aagataccct gaccttccct atgtttatgt gaactcatct 420<br>gggaccattg agaactataa gcccatccag gtggtctctg cgtgcagttt agagacatat 480<br>gcttttccat ttgatgtcca gaattgcagc ctgaccttca agagcattct gcatacagtg 540<br>gaagacgtag acctggcctt tctgaggagc ccagaagaca ttcagcatga caaaaaggcg 600<br>tttttgaatg acagtgagtg ggaacttcta tctgtgtcct ccacatacag catcctgcag 660<br>agcagcgctg gaggatttgc acagattcag tttaatgtgg tgatgcgcag gcacccccctg 720<br>gtctatgtcg tgagtctgct gattcctagc atctttctca tgctggtgga cctggggagc 780<br>ttctacctgc cacccaactg ccgagccagg attgtgttca agaccagtgt gctggtgggc 840<br>tacaccgtct tcagggtcaa catgtccaac caggtgccac ggagtgtagg gagcacccct 900<br>ctgattgggc acttcttcac catctgcatg gccttcttgg ttctcagctt agctaagtcc 960<br>atcgtgttgg tcaaattcct ccatgatgag cagcgtggtg gacaggagca gcccttcttg 1020<br>tgccttcgag gggacaccga tgctgacagg cctagagtgg aacccagggc ccaacgtgct 1080<br>gtggtaacag agtcctcgct gtatggagag cacctggccc agccaggaac cctgaaggaa 1140<br>gtctggtcgc agcttcaatc tatcagcaac tacctccaaa ctcaggacca gacagaccaa 1200<br>caggaggcag agtggctggt cctcctgtcc cgctttgacc gactgctctt ccaaagctac 1260<br>cttttcatgc tggggatcta caccatcact ctgtgctccc tctgggcact gtggggcggc 1320<br>gtgtgaagac tgaagtgttc ttcagtaatt gtgctggcac ttaggagaga gaggaggggg 1380<br>aataatagtg ggttaaaaag ctttctgggt cgggtgtggt ggttcttgcc tatagtccca 1440<br>gtgctttggg aggccatagc aggaggattg cttgagccca ggagttcgag accagccaga 1500<br>gcaacatagt gagaccacat ctctaccagt aaataaataa ataaataaat aaataaataa 1560<br>ataaataaat agctgggcat agtggctcat gcctgtactc tcagctactt gggaggttga 1620<br>ggtgggagga ttgcttgagc ccaggatttc aaggctgcag tgagccatga ttgcaccact 1680<br>gcaccccagc ctgggtgaca gagcaagacc ctgtctcaaa aaaaataaaa taaaaggctt 1740<br>tctgccttca aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 1780 |
| **3-57** | **Sequences** | |
| 3-57-1 | Sequence Number [ID] | 57 |
| 3-57-2 | Molecule Type | AA |
| 3-57-3 | Length | 441 |
| 3-57-4 | Features Location/ | **source 1..441** |
| | Qualifiers | mol_type=protein |
| | | organism=Homo sapiens |
| | NonEnglishQualifier Value | |
| 3-57-5 | Residues | MLSSVMAPLW ACILVAAGIL ATDTHHPQDS ALYHLSKQLL QKYHKEVRPV YNWTKATTVY 60<br>LDLFVHAILD VDAENQILKT SVWYQEVWND EFLSWNSSMF DEIREISLPL SAIWAPDIII 120<br>NEFVDIERYP DLPYVYVNSS GTIENYKPIQ VVSACSLETY AFPFDVQNCS LTFKSILHTV 180<br>EDVDLAFLRS PEDIQHDKKA FLNDSEWELL SVSSTYSILQ SSAGGFAQIQ FNVVMRRHPL 240<br>VYVVSLLIPS IFLMLVDLGS FYLPPNCRAR IVFKTSVLVG YTVFRVNMSN QVPRSVGSTP 300<br>LIGHFFTICM AFLVLSLAKS IVLVKFLHDE QRGGQEQPFL CLRGDTDADR PRVEPRAQRA 360<br>VVTESSLYGE HLAQPGTLKE VWSQLQSISN YLQTQDQTDQ QEAEWLVLLS RFDRLLFQSY 420<br>LFMLGIYTIT LCSLWALWGG V 441 |
| **3-58** | **Sequences** | |
| 3-58-1 | Sequence Number [ID] | 58 |
| 3-58-2 | Molecule Type | AA |
| 3-58-3 | Length | 439 |
| 3-58-4 | Features Location/ | **REGION 1..439** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..439** |
| | | mol_type=protein |
| | | organism=synthetic construct |

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|---|---|---|
| | NonEnglishQualifier Value | |
| 3-58-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDEKNQ VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV RFPDGQIWKP DILLYNSADE 120<br>RFDATFHTNV DVNSSGHCQY LPPGIFKSSC PMDLKNFPMD VQTCKLKFGS WDYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFY ECCKEPYPDV TFTVTMRRRM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS RASLPKVSYV KAIDIWMAVC 300<br>LLFVFSALLE YAAVNFVSRQ HKELLRFRRK RRHHKSPMLN LFQEDEAGEG RFNFSAYGMG 360<br>PACLQAKDGI SVKGANNSNT TNPPPAPSKS PEEMRKLFIQ RAKKIDKISR IGFPMAFLIF 420<br>NMFYWIIYKI VRREDVHNQ 439 |
| **3-59** | **Sequences** | |
| 3-59-1 | Sequence Number [ID] | 59 |
| 3-59-2 | Molecule Type | AA |
| 3-59-3 | Length | 439 |
| 3-59-4 | Features Location/ | **REGION 1..439** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..439** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-59-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDEKNQ VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV RFPDGQIWKP DILLYNSADE 120<br>RFDATFHTNV SVNSSGHCQY LPPGIFKSSC PMDLKNFPMD VQTCKLKFGS WDYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFY ECCKEPYPDV TFTVTMRRRM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS RASLPKVSYV KAIDIWMAVC 300<br>LLFVFSALLE YAAVNFVSRQ HKELLRFRRK RRHHKSPMLN LFQEDEAGEG RFNFSAYGMG 360<br>PACLQAKDGI SVKGANNSNT TNPPPAPSKS PEEMRKLFIQ RAKKIDKISR IGFPMAFLIF 420<br>NMFYWIIYKI VRREDVHNQ 439 |
| **3-60** | **Sequences** | |
| 3-60-1 | Sequence Number [ID] | 60 |
| 3-60-2 | Molecule Type | AA |
| 3-60-3 | Length | 439 |
| 3-60-4 | Features Location/ | **REGION 1..439** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..439** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-60-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDEKNQ VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV RFPDGQIWKP DILLYNSADE 120<br>RFDATFHTNV TVNSSGHCQY LPPGIFKSSC PMDLKNFPMD VQTCKLKFGS WDYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFY ECCKEPYPDV TFTVTMRRRM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS RASLPKVSYV KAIDIWMAVC 300<br>LLFVFSALLE YAAVNFVSRQ HKELLRFRRK RRHHKSPMLN LFQEDEAGEG RFNFSAYGMG 360<br>PACLQAKDGI SVKGANNSNT TNPPPAPSKS PEEMRKLFIQ RAKKIDKISR IGFPMAFLIF 420<br>NMFYWIIYKI VRREDVHNQ 439 |
| **3-61** | **Sequences** | |
| 3-61-1 | Sequence Number [ID] | 61 |
| 3-61-2 | Molecule Type | AA |
| 3-61-3 | Length | 439 |
| 3-61-4 | Features Location/ | **REGION 1..439** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..439** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-61-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDEKNQ VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV RFPDGQIWKP DILLDNSADE 120<br>RFDATFHTNV EVNSSGHCQY LPPGIFKSSC PMDLKNFPMD VQTCKLKFGS WSYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFY ECCKEPYPDV TFTVTMRRRM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS RASLPKVSYV KAIDIWMAVC 300<br>LLFVFSALLE YAAVNFVSRQ HKELLRFRRK RRHHKSPMLN LFQEDEAGEG RFNFSAYGMG 360<br>PACLQAKDGI SVKGANNSNT TNPPPAPSKS PEEMRKLFIQ RAKKIDKISR IGFPMAFLIF 420<br>NMFYWIIYKI VRREDVHNQ 439 |
| **3-62** | **Sequences** | |
| 3-62-1 | Sequence Number [ID] | 62 |

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| 3-62-2 | Molecule Type | AA |
| 3-62-3 | Length | 439 |
| 3-62-4 | Features Location/ | **REGION 1..439** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..439** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-62-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDEKNQ VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV RFPDGQIWKP DILLDNSADE 120<br>RFDATFHTNV QVNSSGHCQY LPPGIFKSSC PMDLKNFPMD VQTCKLKFGS WSYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFY ECCKEPYPDV TFTVTMRRRM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS RASLPKVSYV KAIDIWMAVC 300<br>LLFVFSALLE YAAVNFVSRQ HKELLRFRRK RRHHKSPMLN LFQEDEAGEG RFNFSAYGMG 360<br>PACLQAKDGI SVKGANNSNT TNPPPAPSKS PEEMRKLFIQ RAKKIDKISR IGFPMAFLIF 420<br>NMFYWIIYKI VRREDVHNQ 439 |
| **3-63** | **Sequences** | |
| 3-63-1 | Sequence Number [ID] | 63 |
| 3-63-2 | Molecule Type | AA |
| 3-63-3 | Length | 439 |
| 3-63-4 | Features Location/ | **REGION 1..439** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..439** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-63-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDEKNQ VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV RFPDGQIWKP DILLDNSADE 120<br>RFDATFHTNV LVNSSGHCQY LPPGIFKSSC PMDLKNFPMD VQTCKLKFGT WSYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFY ECCKEPYPDV TFTVTMRRRM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS RASLPKVSYV KAIDIWMAVC 300<br>LLFVFSALLE YAAVNFVSRQ HKELLRFRRK RRHHKSPMLN LFQEDEAGEG RFNFSAYGMG 360<br>PACLQAKDGI SVKGANNSNT TNPPPAPSKS PEEMRKLFIQ RAKKIDKISR IGFPMAFLIF 420<br>NMFYWIIYKI VRREDVHNQ 439 |
| **3-64** | **Sequences** | |
| 3-64-1 | Sequence Number [ID] | 64 |
| 3-64-2 | Molecule Type | AA |
| 3-64-3 | Length | 439 |
| 3-64-4 | Features Location/ | **REGION 1..439** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..439** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-64-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDEKNQ VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV RFPDGQIWKP DILLYNSADE 120<br>RFDATFHTNV LVNSSGHCQI LPPGIFKSSC PMDLKNFPMD VQTCKLKFGS WSYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFY ECCKEPYPDV TFTVTMRRRM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS RASLPKVSYV KAIDIWMAVC 300<br>LLFVFSALLE YAAVNFVSRQ HKELLRFRRK RRHHKSPMLN LFQEDEAGEG RFNFSAYGMG 360<br>PACLQAKDGI SVKGANNSNT TNPPPAPSKS PEEMRKLFIQ RAKKIDKISR IGFPMAFLIF 420<br>NMFYWIIYKI VRREDVHNQ 439 |
| **3-65** | **Sequences** | |
| 3-65-1 | Sequence Number [ID] | 65 |
| 3-65-2 | Molecule Type | AA |
| 3-65-3 | Length | 439 |
| 3-65-4 | Features Location/ | **REGION 1..439** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..439** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-65-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDEKNQ VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV FFPDGQIWKP DILLYNSADE 120<br>RFDATFHTNV GVNSSGHCQY LPPGIFKSSC PMDLKNFPMD VQTCKLKFGS WSYGGWSLDL 180 |

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| | | QMQEADISGY IPNGEWDLVG IPGKRSERFY ECCKEPYPDV TFTVTMRRRM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS RASLPKVSYV KAIDIWMAVC 300<br>LLFVFSALLE YAAVNFVSRQ HKELLRFRRK RRHHKSPMLN LFQEDEAGEG RFNFSAYGMG 360<br>PACLQAKDGI SVKGANNSNT TNPPPAPSKS PEEMRKLFIQ RAKKIDKISR IGFPMAFLIF 420<br>NMFYWIIYKI VRREDVHNQ 439 |
| **3-66** | **Sequences** | |
| 3-66-1 | Sequence Number [ID] | 66 |
| 3-66-2 | Molecule Type | AA |
| 3-66-3 | Length | 439 |
| 3-66-4 | Features Location/ | **REGION 1..439** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..439** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-66-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDEKNQ VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV FFPDGQIWKP DILLYNSADE 120<br>RFDATFHTNV DVNSSGHCQY LPPGIFKSSC PMDLKNFPMD VQTCKLKFGS WSYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFY ECCKEPYPDV TFTVTMRRRM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS RASLPKVSYV KAIDIWMAVC 300<br>LLFVFSALLE YAAVNFVSRQ HKELLRFRRK RRHHKSPMLN LFQEDEAGEG RFNFSAYGMG 360<br>PACLQAKDGI SVKGANNSNT TNPPPAPSKS PEEMRKLFIQ RAKKIDKISR IGFPMAFLIF 420<br>NMFYWIIYKI VRREDVHNQ 439 |
| **3-67** | **Sequences** | |
| 3-67-1 | Sequence Number [ID] | 67 |
| 3-67-2 | Molecule Type | AA |
| 3-67-3 | Length | 439 |
| 3-67-4 | Features Location/ | **REGION 1..439** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..439** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-67-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDEKNQ VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV RFPDGQIWKP DILLENSADE 120<br>RFDATFHTNV LVNSSGHCQY LPPGIFKSSC PMDLKNFPMD VQTCKLKFGS WSYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFW ECCKEPYPDV TFTVTMRRRM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS RASLPKVSYV KAIDIWMAVC 300<br>LLFVFSALLE YAAVNFVSRQ HKELLRFRRK RRHHKSPMLN LFQEDEAGEG RFNFSAYGMG 360<br>PACLQAKDGI SVKGANNSNT TNPPPAPSKS PEEMRKLFIQ RAKKIDKISR IGFPMAFLIF 420<br>NMFYWIIYKI VRREDVHNQ 439 |
| **3-68** | **Sequences** | |
| 3-68-1 | Sequence Number [ID] | 68 |
| 3-68-2 | Molecule Type | AA |
| 3-68-3 | Length | 439 |
| 3-68-4 | Features Location/ | **REGION 1..439** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..439** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-68-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDEKNQ VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV WFPDGQIWKP DILLYNSADE 120<br>RFDATFHTNV LVNSSGHCQY LPPGIFKSSC PMDLKNFPMD VQTCKLKFGS WSYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFV ECCKEPYPDV TFTVTMRRRM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS RASLPKVSYV KAIDIWMAVC 300<br>LLFVFSALLE YAAVNFVSRQ HKELLRFRRK RRHHKSPMLN LFQEDEAGEG RFNFSAYGMG 360<br>PACLQAKDGI SVKGANNSNT TNPPPAPSKS PEEMRKLFIQ RAKKIDKISR IGFPMAFLIF 420<br>NMFYWIIYKI VRREDVHNQ 439 |
| **3-69** | **Sequences** | |
| 3-69-1 | Sequence Number [ID] | 69 |
| 3-69-2 | Molecule Type | AA |
| 3-69-3 | Length | 439 |
| 3-69-4 | Features Location/ | **REGION 1..439** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |

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|---|---|---|
| | | **source 1..439** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-69-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDEKNQ VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV FFPDGQIWKP DILLYNSADE 120<br>RFDATFHTNV LVNSSGHCQY LPPGIFKSSC PMDLKNFPMD VQTCKLKFGS WSYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFV ECCKEPYPDV TFTVTMRRRM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS RASLPKVSYV KAIDIWMAVC 300<br>LLFVFSALLE YAAVNFVSRQ HKELLRFRRK RRHHKSPMLN LFQEDEAGEG RFNFSAYGMG 360<br>PACLQAKDGI SVKGANNSNT TNPPPAPSKS PEEMRKLFIQ RAKKIDKISR IGFPMAFLIF 420<br>NMFYWIIYKI VRREDVHNQ 439 |
| **3-70** | **Sequences** | |
| 3-70-1 | Sequence Number [ID] | 70 |
| 3-70-2 | Molecule Type | AA |
| 3-70-3 | Length | 439 |
| 3-70-4 | Features Location/ | **REGION 1..439** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..439** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-70-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDEKNQ VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV FFPDGQIWKP DILLYNSADE 120<br>RFDATFHTNV LVNSSGHCQY LPPGIFKSSC PMDLKNFPMD VQTCKLKFGS WSYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFF ECCKEPYPDV TFTVTMRRRM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS RASLPKVSYV KAIDIWMAVC 300<br>LLFVFSALLE YAAVNFVSRQ HKELLRFRRK RRHHKSPMLN LFQEDEAGEG RFNFSAYGMG 360<br>PACLQAKDGI SVKGANNSNT TNPPPAPSKS PEEMRKLFIQ RAKKIDKISR IGFPMAFLIF 420<br>NMFYWIIYKI VRREDVHNQ 439 |
| **3-71** | **Sequences** | |
| 3-71-1 | Sequence Number [ID] | 71 |
| 3-71-2 | Molecule Type | AA |
| 3-71-3 | Length | 439 |
| 3-71-4 | Features Location/ | **REGION 1..439** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..439** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-71-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDEKNQ VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV MFPDGQIWKP DILLYNSADE 120<br>RFDATFHTNV AVNSSGHCQY LPPGIFKSSC PMDLKNFPMD VQTCKLKFGS WSYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFY ECCKEPYPDV TFTVTMRRRM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS RASLPKVSYV KAIDIWMAVC 300<br>LLFVFSALLE YAAVNFVSRQ HKELLRFRRK RRHHKSPMLN LFQEDEAGEG RFNFSAYGMG 360<br>PACLQAKDGI SVKGANNSNT TNPPPAPSKS PEEMRKLFIQ RAKKIDKISR IGFPMAFLIF 420<br>NMFYWIIYKI VRREDVHNQ 439 |
| **3-72** | **Sequences** | |
| 3-72-1 | Sequence Number [ID] | 72 |
| 3-72-2 | Molecule Type | AA |
| 3-72-3 | Length | 439 |
| 3-72-4 | Features Location/ | **REGION 1..439** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..439** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-72-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDEKNQ VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV MFPDGQIWKP DILLYNSADE 120<br>RFDATFHTNV FVNSSGHCQY LPPGIFKSSC PMDLKNFPMD VQTCKLKFGS WSYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFY ECCKEPYPDV TFTVTMRRRM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS RASLPKVSYV KAIDIWMAVC 300<br>LLFVFSALLE YAAVNFVSRQ HKELLRFRRK RRHHKSPMLN LFQEDEAGEG RFNFSAYGMG 360 |

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| | | PACLQAKDGI SVKGANNSNT TNPPPAPSKS PEEMRKLFIQ RAKKIDKISR IGFPMAFLIF 420<br>NMFYWIIYKI VRREDVHNQ 439 |
| **3-73** | **Sequences** | |
| 3-73-1 | Sequence Number [ID] | 73 |
| 3-73-2 | Molecule Type | AA |
| 3-73-3 | Length | 439 |
| 3-73-4 | Features Location/ | **REGION 1..439** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..439** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-73-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDEKNQ VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV WFPDGQIWKP DILLENSADE 120<br>RFDATFHTNV LVNSSGHCQY LPPGIFKSSC PMDLKNFPMD VQTCKLKFGS WSYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFW ECCKEPYPDV TFTVTMRRRM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS RASLPKVSYV KAIDIWMAVC 300<br>LLFVFSALLE YAAVNFVSRQ HKELLRFRRK RRHHKSPMLN LFQEDEAGEG RFNFSAYGMG 360<br>PACLQAKDGI SVKGANNSNT TNPPPAPSKS PEEMRKLFIQ RAKKIDKISR IGFPMAFLIF 420<br>NMFYWIIYKI VRREDVHNQ 439 |
| **3-74** | **Sequences** | |
| 3-74-1 | Sequence Number [ID] | 74 |
| 3-74-2 | Molecule Type | AA |
| 3-74-3 | Length | 439 |
| 3-74-4 | Features Location/ | **REGION 1..439** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..439** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-74-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDEKNQ VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV FFPDGQIWKP DILLENSADE 120<br>RFDATFHTNV LVNSSGHCQY LPPGIFKSSC PMDLKNFPMD VQTCKLKFGS WSYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFW ECCKEPYPDV TFTVTMRRRM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS RASLPKVSYV KAIDIWMAVC 300<br>LLFVFSALLE YAAVNFVSRQ HKELLRFRRK RRHHKSPMLN LFQEDEAGEG RFNFSAYGMG 360<br>PACLQAKDGI SVKGANNSNT TNPPPAPSKS PEEMRKLFIQ RAKKIDKISR IGFPMAFLIF 420<br>NMFYWIIYKI VRREDVHNQ 439 |
| **3-75** | **Sequences** | |
| 3-75-1 | Sequence Number [ID] | 75 |
| 3-75-2 | Molecule Type | AA |
| 3-75-3 | Length | 439 |
| 3-75-4 | Features Location/ | **REGION 1..439** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..439** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-75-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDEKNQ VLTTNIFLQM SWTDHYLQWN VSEYPGVKTV FFPDGQIWKP DILLYNSADE 120<br>RFDATFHTNV DVNSSGHCQY LPPGIFKSSC PMDLKNFPMD VQTCKLKFGS WSYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFY ECCKEPYPDV TFTVTMRRRM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS RASLPKVSYV KAIDIWMAVC 300<br>LLFVFSALLE YAAVNFVSRQ HKELLRFRRK RRHHKSPMLN LFQEDEAGEG RFNFSAYGMG 360<br>PACLQAKDGI SVKGANNSNT TNPPPAPSKS PEEMRKLFIQ RAKKIDKISR IGFPMAFLIF 420<br>NMFYWIIYKI VRREDVHNQ 439 |
| **3-76** | **Sequences** | |
| 3-76-1 | Sequence Number [ID] | 76 |
| 3-76-2 | Molecule Type | AA |
| 3-76-3 | Length | 439 |
| 3-76-4 | Features Location/ | **REGION 1..439** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..439** |
| | | mol_type=protein |
| | | organism=synthetic construct |

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| | NonEnglishQualifier Value | |
| 3-76-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDEKNQ VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV FFPDGQIWKP DILLYNSADE 120<br>RFDATFHTNV NVNSSGHCQY LPPGIFKSSC PMDLKNFPMD VQTCKLKFGS WDYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFY ECCKEPYPDV TFTVTMRRRM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS RASLPKVSYV KAIDIWMAVC 300<br>LLFVFSALLE YAAVNFVSRQ HKELLRFRRK RRHHKSPMLN LFQEDEAGEG RFNFSAYGMG 360<br>PACLQAKDGI SVKGANNSNT TNPPPAPSKS PEEMRKLFIQ RAKKIDKISR IGFPMAFLIF 420<br>NMFYWIIYKI VRREDVHNQ 439 |
| **3-77** | **Sequences** | |
| 3-77-1 | Sequence Number [ID] | 77 |
| 3-77-2 | Molecule Type | AA |
| 3-77-3 | Length | 439 |
| 3-77-4 | Features Location/ | **REGION 1..439** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..439** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-77-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDEKNQ VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV RFPDGQIWKP DILLYNSADE 120<br>RFDATFHTNV LVNSSGHCEY LPPGIFKSSC PMDLKNFPMD VQTCKLKFGS WDYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFY ECCKEPYPDV TFTVTMRRRM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS RASLPKVSYV KAIDIWMAVC 300<br>LLFVFSALLE YAAVNFVSRQ HKELLRFRRK RRHHKSPMLN LFQEDEAGEG RFNFSAYGMG 360<br>PACLQAKDGI SVKGANNSNT TNPPPAPSKS PEEMRKLFIQ RAKKIDKISR IGFPMAFLIF 420<br>NMFYWIIYKI VRREDVHNQ 439 |
| **3-78** | **Sequences** | |
| 3-78-1 | Sequence Number [ID] | 78 |
| 3-78-2 | Molecule Type | AA |
| 3-78-3 | Length | 439 |
| 3-78-4 | Features Location/ | **REGION 1..439** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..439** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-78-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDEKNQ VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV RFPDGQIWKP DILLYNSADE 120<br>RFDATFHTNV LVNSSGHCQY LPPGIFKSSC PMDLKNFPMD VQTCKLKFGS WDYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFW ECCKEPYPDV TFTVTMRRRM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS RASLPKVSYV KAIDIWMAVC 300<br>LLFVFSALLE YAAVNFVSRQ HKELLRFRRK RRHHKSPMLN LFQEDEAGEG RFNFSAYGMG 360<br>PACLQAKDGI SVKGANNSNT TNPPPAPSKS PEEMRKLFIQ RAKKIDKISR IGFPMAFLIF 420<br>NMFYWIIYKI VRREDVHNQ 439 |
| **3-79** | **Sequences** | |
| 3-79-1 | Sequence Number [ID] | 79 |
| 3-79-2 | Molecule Type | AA |
| 3-79-3 | Length | 724 |
| 3-79-4 | Features Location/ | **REGION 1..724** |
| | Qualifiers | note=AAV5 capsid protein |
| | | **source 1..724** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-79-5 | Residues | MSFVDHPPDW LEEVGEGLRE FLGLEAGPPK PKPNQQHQDQ ARGLVLPGYN YLGPGNGLDR 60<br>GEPVNRADEV AREHDISYNE QLEAGDNPYL KYNHADAEFQ EKLADDTSFG GNLGKAVFQA 120<br>KKRVLEPFGL VEEGAKTAPT GKRIDDHFPK RKKARTEEDS KPSTSSDAEA GPSGSQQLQI 180<br>PAQPASSLGA DTMSAGGGGP LGDNNQGADG VGNASGDWHC DSTWMGDRVV TKSTRTWVLP 240<br>SYNNHQYREI KSGSVDGSNA NAYFGYSTPW GYFDFNRFHS HWSPRDWQRL INNYWGFRPR 300<br>SLRVKIFNIQ VKEVTVQDST TTIANNLTST VQVFTDDDYQ LPYVVGNGTE GCLPAFPPQV 360<br>FTLPQYGYAT LNRDNTENPT ERSSFFCLEY FPSKMLRTGN NFEFTYNFEE VPFHSSFAPS 420<br>QNLFKLANPL VDQYLYRFVS TNNTGGVQFN KNLAGRYANT YKNWFPGPMG RTQGWNLGSG 480<br>VNRASVSAFA TTNRMELEGA SYQVPPQPNG MTNNLQGSNT YALENTMIFN SQPANPGTTA 540<br>TYLEGNMLIT SESETQPVNR VAYNVGGQMA TNNQSSTTAP ATGTYNLQEI VPGSVWMERD 600 |

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| | | VYLQGPIWAK IPETGAHFHP SPAMGGFGLK HPPPMMLIKN TPVPGNITSF SDVPVSSFIT 660<br>QYSTGQVTVE MEWELKKENS KRWNPEIQYT NNYNDPQFVD FAPDSTGEYR TTRPIGTRYL 720<br>TRPL 724 |
| **3-80** | **Sequences** | |
| 3-80-1 | Sequence Number [ID] | 80 |
| 3-80-2 | Molecule Type | AA |
| 3-80-3 | Length | 736 |
| 3-80-4 | Features Location/ | **REGION 1..736** |
| | Qualifiers | note=AAV6 capsid protein |
| | | **source 1..736** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-80-5 | Residues | MAADGYLPDW LEDNLSEGIR EWWDLKPGAP KPKANQQKQD DGRGLVLPGY KYLGPFNGLD 60<br>KGEPVNAADA AALEHDKAYD QQLKAGDNPY LRYNHADAEF QERLQEDTSF GGNLGRAVFQ 120<br>AKKRVLEPFG LVEEGAKTAP GKKRPVEQSP QEPDSSSGIG KTGQQPAKKR LNFGQTGDSE 180<br>SVPDPQPLGE PPATPAAVGP TTMASGGGAP MADNNEGADG VGNASGNWHC DSTWLGDRVI 240<br>TTSTRTWALP TYNNHLYKQI SSASTGASND NHYFGYSTPW GYFDFNRFHC HFSPRDWQRL 300<br>INNNWGFRPK RLNFKLFNIQ VKEVTTNDGV TTIANNLTST VQVFSDSEYQ LPYVLGSAHQ 360<br>GCLPPFPADV FMIPQYGYLT LNNGSQAVGR SSFYCLEYFP SQMLRTGNNF TFSYTFEDVP 420<br>FHSSYAHSQS LDRLMNPLID QYLYYLNRTQ NQSGSAQNKD LLFSRGSPAG MSVQPKNWLP 480<br>GPCYRQQRVS KTKTDNNNSN FTWTGASKYN LNGRESIINP GTAMASHKDD KDKFFPMSGV 540<br>MIFGKESAGA SNTALDNVMI TDEEEIKATN PVATERFGTV AVNLQSSSTD PATGDVHVMG 600<br>ALPGMVWQDR DVYLQGPIWA KIPHTDGHFH PSPLMGGFGL KHPPPQILIK NTPVPANPPA 660<br>EFSATKFASF ITQYSTGQVS VEIEWELQKE NSKRWNPEVQ YTSNYAKSAN VDFTVDNNGL 720<br>YTEPRPIGTR YLTRPL 736 |
| **3-81** | **Sequences** | |
| 3-81-1 | Sequence Number [ID] | 81 |
| 3-81-2 | Molecule Type | AA |
| 3-81-3 | Length | 736 |
| 3-81-4 | Features Location/ | **REGION 1..736** |
| | Qualifiers | note=AAV9 capsid protein |
| | | **source 1..736** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-81-5 | Residues | MAADGYLPDW LEDNLSEGIR EWWALKPGAP QPKANQQHQD NARGLVLPGY KYLGPGNGLD 60<br>KGEPVNAADA AALEHDKAYD QQLKAGDNPY LKYNHADAEF QERLKEDTSF GGNLGRAVFQ 120<br>AKKRLLEPLG LVEEAAKTAP GKKRPVEQSP QEPDSSAGIG KSGAQPAKKR LNFGQTGDTE 180<br>SVPDPQPIGE PPAAPSGVGS LTMASGGGAP VADNNEGADG VGSSSGNWHC DSQWLGDRVI 240<br>TTSTRTWALP TYNNHLYKQI SNSTSGGSSN DNAYFGYSTP WGYFDFNRFH CHFSPRDWQR 300<br>LINNNWGFRP KRLNFKLFNI QVKEVTDNNG VKTIANNLTS TVQVFTDSDY QLPYVLGSAH 360<br>EGCLPPFPAD VFMIPQYGYL TLNDGSQAVG RSSFYCLEYF PSQMLRTGNN FQFSYEFENV 420<br>PFHSSYAHSQ SLDRLMNPLI DQYLYYLSKT INGSGQNQQT LKFSVAGPSN MAVQGRNYIP 480<br>GPSYRQQRVS TTVTQNNNSE FAWPGASSWA LNGRNSLMNP GPAMASHKEG EDRFFPLSGS 540<br>LIFGKQGTGR DNVDADKVMI TNEEEIKTTN PVATESYGQV ATNHQSAQAQ AQTGWVQNQG 600<br>ILPGMVWQDR DVYLQGPIWA KIPHTDGNFH PSPLMGGFGM KHPPPQILIK NTPVPADPPT 660<br>AFNKDKLNSF ITQYSTGQVS VEIEWELQKE NSKRWNPEIQ YTSNYYKSNN VEFAVNTEGV 720<br>YSEPRPIGTR YLTRNL 736 |
| **3-82** | **Sequences** | |
| 3-82-1 | Sequence Number [ID] | 82 |
| 3-82-2 | Molecule Type | DNA |
| 3-82-3 | Length | 1359 |
| 3-82-4 | Features Location/ | **source 1..1359** |
| | Qualifiers | mol_type=other DNA |
| | | organism=Homo sapiens |
| | NonEnglishQualifier Value | |
| 3-82-5 | Residues | atgaaccggc agctagtgaa cattttgaca gccttgtttg cattttttctt agagacaaac 60<br>cacttcagga cggctttctg caaagaccat gactccaggt ctggaaaaca accttcacag 120<br>accctatctc cttcagattt cttggacaag ttaatgggaa ggacatcagg atatgatgca 180<br>agaatcaggc caaattttaa aggtcctcca gtaaacgtta cttgcaatat ttttatcaac 240<br>agttttggat cagtcacaga aacgaccatg gactaccgag tgaatatttt tctgagacaa 300<br>cagtggaatg attcacggct ggcgtacagt gagtacccag atgactccct ggacttggac 360<br>ccatccatgc tagactccat ttggaaacca gatttgttct ttgccaatga gaagggtgcc 420<br>aacttccacg atgtcaccac tgacaacaaa ttgctacgga tttcgaaaaa tggcaaagtg 480 |

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|---|---|---|
| | | ctctacagta tcagactcac cttgacctta tcctgtccca tggacttgaa gaactttccg 540<br>atggatgtcc agacctgtac aatgcagctg gagagttttg ggtacacgat gaatgacctg 600<br>atatttgagt ggttaagtga tggtccagtg caagttgctg aaggattgac cctgccccag 660<br>tttattttga aagaagagaa ggaacttggc tactgtacaa agcactacaa cactggaaag 720<br>tttacctgca ttgaggtcaa gtttcatctg gaacgccaaa tgggatatta tttgatccag 780<br>atgtacatcc caagcctgct tatagtaatt ttgtcctggg tttccttttg gataaatatg 840<br>gatgcagccc ctgccagggt cgcactgggc atcaccacag tcttaacgat gaccacccag 900<br>agttcaggct ccagggcatc tctgccaaag gtctcctatg taaaagcgat tgacatctgg 960<br>atggcggtgt gccttctgtt tgtgtttgct gccttactgg aatacgcagc ggtgaacttc 1020<br>gtctccaggc aacacaagga gttcctgcgc ctccgaagaa gacagaagag gcagaataag 1080<br>gaagaagacg ttactcgtga aagtcgtttt aattttagcg gttatgggat gggtcactgc 1140<br>ctccaagtga aagatggaac agctgtcaag gccacacctg ccaacccact cccacaaccg 1200<br>ccaaaagatg gagatgctat caagaagaag tttgtggacc gggcaaaaag gattgacacg 1260<br>atatctcgag ctgccttccc attggccttc ctcattttca acatctttta ctggatcaca 1320<br>tacaagatca ttcggcatga agatgtccac aagaaatag 1359 |
| **3-83** | **Sequences** | |
| 3-83-1 | Sequence Number [ID] | 83 |
| 3-83-2 | Molecule Type | AA |
| 3-83-3 | Length | 452 |
| 3-83-4 | Features Location/ | **source 1..452** |
| | Qualifiers | mol_type=protein |
| | | organism=Homo sapiens |
| | NonEnglishQualifier Value | |
| 3-83-5 | Residues | MNRQLVNILT ALFAFFLETN HFRTAFCKDH DSRSGKQPSQ TLSPSDFLDK LMGRTSGYDA 60<br>RIRPNFKGPP VNVTCNIFIN SFGSVTETTM DYRVNIFLRQ QWNDSRLAYS EYPDDSLDLD 120<br>PSMLDSIWKP DLFFANEKGA NFHDVTTDNK LLRISKNGKV LYSIRLTLTL SCPMDLKNFP 180<br>MDVQTCTMQL ESFGYTMNDL IFEWLSDGPV QVAEGLTLPQ FILKEEKELG YCTKHYNTGK 240<br>FTCIEVKFHL ERQMGYYLIQ MYIPSLLIVI LSWVSFWINM DAAPARVALG ITTVLTMTTQ 300<br>SSGSRASLPK VSYVKAIDIW MAVCLLFVFA ALLEYAAVNF VSRQHKEFLR LRRRQKRQNK 360<br>EEDVTRESRF NFSGYGMGHC LQVKDGTAVK ATPANPLPQP PKDGDAIKKK FVDRAKRIDT 420<br>ISRAAFPLAF LIFNIFYWIT YKIIRHEDVH KK 452 |
| **3-84** | **Sequences** | |
| 3-84-1 | Sequence Number [ID] | 84 |
| 3-84-2 | Molecule Type | DNA |
| 3-84-3 | Length | 1395 |
| 3-84-4 | Features Location/ | **source 1..1395** |
| | Qualifiers | mol_type=other DNA |
| | | organism=Homo sapiens |
| | NonEnglishQualifier Value | |
| 3-84-5 | Residues | atggcccacg tgagacactt tcggacatta gtttcgggat tttacttctg ggaagcagca 60<br>ctgttactca gtttggttgc cacaaaggaa acagacagtg caagatctcg aagtgctcca 120<br>atgtcacctt ctgattttct ggataaatta atgggcagga catcaggata tgatgcaaga 180<br>atcagaccca attttaaagg ccctccagtt aatgtcacat gcaacatatt catcaacagc 240<br>tttggctcta tcgcagagac gaccatggat tacagagtga atatctttct tcgtcagaaa 300<br>tggaatgatc cccgcctcgc gtacagtgaa tatcctgacg actctttaga cctcgacccc 360<br>tccatgttgg actccatttg gaaacctgat ttgttctttg ccaatgaaaa gggtgccaac 420<br>tttcatgaag tcactacaga caacaaattg ctaagaattt tcaaaaatgg aaatgttctt 480<br>tattcaataa gattaacatt aacactttcc tgtccaatgg atctcaagaa ttttcccatg 540<br>gatgtacaaa catgtataat gcaactggaa agctttgggt acacaatgaa tgatctcatt 600<br>tttgaatggc aagatgaggc acccgtacaa gtggcagaag gactcacttt gccccagttt 660<br>ctgttgaaag aagaaaaaga tttacgatac tgcactaaac attacaatac aggaaagttt 720<br>acgtgtatag aagtgcgatt ccatctggag cgacaaatgg gatactatct gatccagatg 780<br>tacattccca gtctcctgat tgttattcta tcctgggttt cattctggat caacatggat 840<br>gcagcaccgg ccagggtagc tctggggata accactgtgc taacgatgac tacacagagt 900<br>tcaggatcac gagcttcctt gccaaaagtt tcatatgtca aagctattga tatttggatg 960<br>gcagtatgcc tccttttttgt gttttcagca cttctggagt atgcagctgt aaattttgta 1020<br>tcaagacaac acaaagaact tctgagattt cgacgaaaga gaaagaataa gacagaagct 1080<br>tttgcactgg agaagtttta ccgtttctca gatatggatg atgaggtaag ggaaagccga 1140<br>ttcagcttca cagcctatgg aatgggacca tgtctacaag caaaggatgg catgactcca 1200<br>aagggcccca accaccctgt ccaggtaatg ccaaaaagtc ctgatgaaat gaggaaggtc 1260<br>tttatcgacc gggccaagaa gattgatacc atctcccgag cctgcttccc attagctttt 1320<br>ttgattttta atattttcta ctgggttatc tataaaattc ttaggcatga ggatattcat 1380<br>cagcagcaag attaa 1395 |
| **3-85** | **Sequences** | |
| 3-85-1 | Sequence Number [ID] | 85 |

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|---|---|---|
| 3-85-2 | Molecule Type | AA |
| 3-85-3 | Length | 464 |
| 3-85-4 | Features Location/ | **source 1..464** |
| | Qualifiers | mol_type=protein |
| | | organism=Homo sapiens |
| | NonEnglishQualifier Value | |
| 3-85-5 | Residues | MAHVRHFRTL VSGFYFWEAA LLLSLVATKE TDSARSRSAP MSPSDFLDKL MGRTSGYDAR 60<br>IRPNFKGPPV NVTCNIFINS FGSIAETTMD YRVNIFLRQK WNDPRLAYSE YPDDSLDLDP 120<br>SMLDSIWKPD LFFANEKGAN FHEVTTDNKL LRIFKNGNVL YSIRLTLTLS CPMDLKNFPM 180<br>DVQTCIMQLE SFGYTMNDLI FEWQDEAPVQ VAEGLTLPQF LLKEEKDLRY CTKHYNTGKF 240<br>TCIEVRFHLE RQMGYYLIQM YIPSLLIVIL SWVSFWINMD AAPARVALGI TTVLTMTTQS 300<br>SGSRASLPKV SYVKAIDIWM AVCLLFVFSA LLEYAAVNFV SRQHKELLRF RRKRKNKTEA 360<br>FALEKFYRFS DMDDEVRESR FSFTAYGMGP CLQAKDGMTP KGPNHPVQVM PKSPDEMRKV 420<br>FIDRAKKIDT ISRACFPLAF LIFNIFYWVI YKILRHEDIH QQQD 464 |
| **3-86** | **Sequences** | |
| 3-86-1 | Sequence Number [ID] | 86 |
| 3-86-2 | Molecule Type | DNA |
| 3-86-3 | Length | 1350 |
| 3-86-4 | Features Location/ | **source 1..1350** |
| | Qualifiers | mol_type=other DNA |
| | | organism=Homo sapiens |
| | NonEnglishQualifier Value | |
| 3-86-5 | Residues | atggcccacg tgagacactt tcggacatta gtttcgggat tttacttctg ggaagcagca 60<br>ctgttactca gtttggttgc cacaaaggaa acagacagtg caagatctcg aagtgctcca 120<br>atgtcacctt ctgattttct ggataaatta atgggcagga catcaggata tgatgcaaga 180<br>atcagaccca attttaaagg ccctccagtt aatgtcacat gcaacatatt catcaacagc 240<br>tttggctcta tcgcagagac gaccatggat tacagagtga atatctttct tcgtcagaaa 300<br>tggaatgatc cccgcctcgc gtacagtgaa tatcctgacg actctttaga cctcgacccc 360<br>tccatgttgg actccatttg gaaacctgat ttgttctttg ccaatgaaaa gggtgccaac 420<br>tttcatgaag tcactacaga caacaaattg ctaagaattt tcaaaaatgg aaatgttctt 480<br>tattcaataa gattaacatt aacactttcc tgtccaatgg atctcaagaa ttttcccatg 540<br>gatgtacaaa catgtataat gcaactggaa agctttgggt acacaatgaa tgatctcatt 600<br>tttgaatggc aagatgaggc acccgtacaa gtggcagaag gactcacttt gccccagttt 660<br>ctgttgaaag aagaaaaaga tttacgatac tgcactaaac attacaatac aggaaagttt 720<br>acgtgtatag aagtgcgatt ccatctggag cgacaaatgg gatactatct gatccagatg 780<br>tacattccca gtctcctgat tgttattcta tcctgggttt cattctggat caacatggat 840<br>gcagcaccgg ccagggtagc tctggggata accactgtgc taacgatgac tacacagagt 900<br>tcaggatcac gagcttcctt gccaaaagtt tcatatgtca aagctattga tatttggatg 960<br>gcagtatgcc tccttttttgt gttttcagca cttctggagt atgcagctgt aaattttgta 1020<br>tcaagacaac acaaagaact tctgagattt cgacgaaaga gaaagaataa ggatgatgag 1080<br>gtaagggaaa gccgattcag cttcacagcc tatggaatgg gaccatgtct acaagcaaag 1140<br>gatggcatga ctccaaaggg ccccaaccac cctgtccagg taatgccaaa aagtcctgat 1200<br>gaaatgagga aggtctttat cgaccgggcc aagaagattg ataccatctc ccgagcctgc 1260<br>ttcccattag ctttttgat ttttaatatt ttctactggg ttatctataa aattcttagg 1320<br>catgaggata ttcatcagca gcaagattaa 1350 |
| **3-87** | **Sequences** | |
| 3-87-1 | Sequence Number [ID] | 87 |
| 3-87-2 | Molecule Type | AA |
| 3-87-3 | Length | 449 |
| 3-87-4 | Features Location/ | **source 1..449** |
| | Qualifiers | mol_type=protein |
| | | organism=Homo sapiens |
| | NonEnglishQualifier Value | |
| 3-87-5 | Residues | MAHVRHFRTL VSGFYFWEAA LLLSLVATKE TDSARSRSAP MSPSDFLDKL MGRTSGYDAR 60<br>IRPNFKGPPV NVTCNIFINS FGSIAETTMD YRVNIFLRQK WNDPRLAYSE YPDDSLDLDP 120<br>SMLDSIWKPD LFFANEKGAN FHEVTTDNKL LRIFKNGNVL YSIRLTLTLS CPMDLKNFPM 180<br>DVQTCIMQLE SFGYTMNDLI FEWQDEAPVQ VAEGLTLPQF LLKEEKDLRY CTKHYNTGKF 240<br>TCIEVRFHLE RQMGYYLIQM YIPSLLIVIL SWVSFWINMD AAPARVALGI TTVLTMTTQS 300<br>SGSRASLPKV SYVKAIDIWM AVCLLFVFSA LLEYAAVNFV SRQHKELLRF RRKRKNKDDE 360<br>VRESRFSFTA YGMGPCLQAK DGMTPKGPNH PVQVMPKSPD EMRKVFIDRA KKIDTISRAC 420<br>FPLAFLIFNI FYWVIYKILR HEDIHQQQD 449 |
| **3-88** | **Sequences** | |
| 3-88-1 | Sequence Number [ID] | 88 |
| 3-88-2 | Molecule Type | AA |
| 3-88-3 | Length | 439 |

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|---|---|---|
| 3-88-4 | Features Location/ | **REGION 1..439** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..439** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-88-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDEKNQ VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV RFPDGQIWKP DILLYNSADE 120<br>RFDATFHTNV LVNSSGHCQC LPPGIFKSSC PMDLKNFPMD VQTCKLKFGS WSYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFY ECCKEPYPDV TFTVTMRRRM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS RASLPKVSYV KAIDIWMAVC 300<br>LLFVFSALLE YAAVNFVSRQ HKELLRFRRK RRHHKSPMLN LFQEDEAGEG RFNFSAYGMG 360<br>PACLQAKDGI SVKGANNSNT TNPPPAPSKS PEEMRKLFIQ RAKKIDKISR IGFPMAFLIF 420<br>NMFYWIIYKI VRREDVHNQ 439 |
| **3-89** | **Sequences** | |
| 3-89-1 | Sequence Number [ID] | 89 |
| 3-89-2 | Molecule Type | AA |
| 3-89-3 | Length | 439 |
| 3-89-4 | Features Location/ | **REGION 1..439** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..439** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-89-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDEKNQ VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV RFPDGQIWKP DILLYNSADE 120<br>RFDATFHTNV LVNSSGHCQR LPPGIFKSSC PMDLKNFPMD VQTCKLKFGS WSYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFY ECCKEPYPDV TFTVTMRRRM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS RASLPKVSYV KAIDIWMAVC 300<br>LLFVFSALLE YAAVNFVSRQ HKELLRFRRK RRHHKSPMLN LFQEDEAGEG RFNFSAYGMG 360<br>PACLQAKDGI SVKGANNSNT TNPPPAPSKS PEEMRKLFIQ RAKKIDKISR IGFPMAFLIF 420<br>NMFYWIIYKI VRREDVHNQ 439 |
| **3-90** | **Sequences** | |
| 3-90-1 | Sequence Number [ID] | 90 |
| 3-90-2 | Molecule Type | AA |
| 3-90-3 | Length | 439 |
| 3-90-4 | Features Location/ | **REGION 1..439** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..439** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-90-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDEKNQ VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV RFPDGQIWKP DILLENSADE 120<br>RFDATFHTNV MVNSSGHCQY LPPGIFKSSC PMDLKNFPMD VQTCKLKFGS WSYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFY ECCKEPYPDV TFTVTMRRRM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS RASLPKVSYV KAIDIWMAVC 300<br>LLFVFSALLE YAAVNFVSRQ HKELLRFRRK RRHHKSPMLN LFQEDEAGEG RFNFSAYGMG 360<br>PACLQAKDGI SVKGANNSNT TNPPPAPSKS PEEMRKLFIQ RAKKIDKISR IGFPMAFLIF 420<br>NMFYWIIYKI VRREDVHNQ 439 |
| **3-91** | **Sequences** | |
| 3-91-1 | Sequence Number [ID] | 91 |
| 3-91-2 | Molecule Type | AA |
| 3-91-3 | Length | 439 |
| 3-91-4 | Features Location/ | **REGION 1..439** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..439** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-91-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDEKNQ VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV RFPDGQIWKP DILLYNSADE 120<br>RFDATFHTNV LVNSSGHCWY LPPGIFKSSC PMDLKNFPMD VQTCKLKFGS WDYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFY ECCKEPYPDV TFTVTMRRRM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS RASLPKVSYV KAIDIWMAVC 300 |

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|---|---|---|
| | | LLFVFSALLE YAAVNFVSRQ HKELLRFRRK RRHHKSPMLN LFQEDEAGEG RFNFSAYGMG 360<br>PACLQAKDGI SVKGANNSNT TNPPPAPSKS PEEMRKLFIQ RAKKIDKISR IGFPMAFLIF 420<br>NMFYWIIYKI VRREDVHNQ 439 |
| **3-92** | **Sequences** | |
| 3-92-1 | Sequence Number [ID] | 92 |
| 3-92-2 | Molecule Type | AA |
| 3-92-3 | Length | 439 |
| 3-92-4 | Features Location/ | **REGION 1..439** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..439** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-92-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDEKNQ VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV RFPDGQIWKP DILLYNSADE 120<br>RFDATFHTNV LVNSSGHCWY LPPGIFKSSC PMDLKNFPMD VQTCKLKFGS WSYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFW ECCKEPYPDV TFTVTMRRRM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS RASLPKVSYV KAIDIWMAVC 300<br>LLFVFSALLE YAAVNFVSRQ HKELLRFRRK RRHHKSPMLN LFQEDEAGEG RFNFSAYGMG 360<br>PACLQAKDGI SVKGANNSNT TNPPPAPSKS PEEMRKLFIQ RAKKIDKISR IGFPMAFLIF 420<br>NMFYWIIYKI VRREDVHNQ 439 |
| **3-93** | **Sequences** | |
| 3-93-1 | Sequence Number [ID] | 93 |
| 3-93-2 | Molecule Type | AA |
| 3-93-3 | Length | 439 |
| 3-93-4 | Features Location/ | **REGION 1..439** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..439** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-93-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDEKNQ VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV RFPDGQIWKP DILLYNSADE 120<br>RFDATFHTNV LVNSSGHCWY LPPGIFKSSC PMDLKNFPMD VQTCKLKFGS WDYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFY ECCKEPIPDV TFTVTMRRRM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS RASLPKVSYV KAIDIWMAVC 300<br>LLFVFSALLE YAAVNFVSRQ HKELLRFRRK RRHHKSPMLN LFQEDEAGEG RFNFSAYGMG 360<br>PACLQAKDGI SVKGANNSNT TNPPPAPSKS PEEMRKLFIQ RAKKIDKISR IGFPMAFLIF 420<br>NMFYWIIYKI VRREDVHNQ 439 |
| **3-94** | **Sequences** | |
| 3-94-1 | Sequence Number [ID] | 94 |
| 3-94-2 | Molecule Type | AA |
| 3-94-3 | Length | 439 |
| 3-94-4 | Features Location/ | **REGION 1..439** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..439** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-94-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDEKNQ VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV RFPDGQIWKP DILLENSADE 120<br>RFDATFHTNV LVNSSGHCEY LPPGIFKSSC PMDLKNFPMD VQTCKLKFGS WDYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFY ECCKEPYPDV TFTVTMRRRM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS RASLPKVSYV KAIDIWMAVC 300<br>LLFVFSALLE YAAVNFVSRQ HKELLRFRRK RRHHKSPMLN LFQEDEAGEG RFNFSAYGMG 360<br>PACLQAKDGI SVKGANNSNT TNPPPAPSKS PEEMRKLFIQ RAKKIDKISR IGFPMAFLIF 420<br>NMFYWIIYKI VRREDVHNQ 439 |
| **3-95** | **Sequences** | |
| 3-95-1 | Sequence Number [ID] | 95 |
| 3-95-2 | Molecule Type | AA |
| 3-95-3 | Length | 439 |
| 3-95-4 | Features Location/ | **REGION 1..439** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..439** |
| | | mol_type=protein |

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|---|---|---|
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-95-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDEKNQ VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV FFPDGQIWKP DILLYNSADE 120<br>RFDATFHTNV GVNSSGHCLY LPPGIFKSSC PMDLKNFPMD VQTCKLKFGS WSYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFY ECCKEPFPDV TFTVTMRRRM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS RASLPKVSYV KAIDIWMAVC 300<br>LLFVFSALLE YAAVNFVSRQ HKELLRFRRK RRHHKSPMLN LFQEDEAGEG RFNFSAYGMG 360<br>PACLQAKDGI SVKGANNSNT TNPPPAPSKS PEEMRKLFIQ RAKKIDKISR IGFPMAFLIF 420<br>NMFYWIIYKI VRREDVHNQ 439 |
| **3-96** | **Sequences** | |
| 3-96-1 | Sequence Number [ID] | 96 |
| 3-96-2 | Molecule Type | AA |
| 3-96-3 | Length | 439 |
| 3-96-4 | Features Location/ | **REGION 1..439** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..439** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-96-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDEKNQ VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV RFPDGQIWKP DILLQNSADE 120<br>RFDATFHTNV LVNSSGHCQY LPPGIFKSSC PMDLKNFPMD VQTCKLKFGS WDYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFY ECCKEPYPDV TFTVTMRRRM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS RASLPKVSYV KAIDIWMAVC 300<br>LLFVFSALLE YAAVNFVSRQ HKELLRFRRK RRHHKSPMLN LFQEDEAGEG RFNFSAYGMG 360<br>PACLQAKDGI SVKGANNSNT TNPPPAPSKS PEEMRKLFIQ RAKKIDKISR IGFPMAFLIF 420<br>NMFYWIIYKI VRREDVHNQ 439 |
| **3-97** | **Sequences** | |
| 3-97-1 | Sequence Number [ID] | 97 |
| 3-97-2 | Molecule Type | AA |
| 3-97-3 | Length | 439 |
| 3-97-4 | Features Location/ | **REGION 1..439** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..439** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-97-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDEKNQ VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV RFPDGQIWKP DILLYNSADE 120<br>RFDATFHTNV LVNSSGHCQY MPPGIFKSSC PMDLKNFPMD VQTCKLKFGS WDYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFW ECCKEPYPDV TFTVTMRRRM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS RASLPKVSYV KAIDIWMAVC 300<br>LLFVFSALLE YAAVNFVSRQ HKELLRFRRK RRHHKSPMLN LFQEDEAGEG RFNFSAYGMG 360<br>PACLQAKDGI SVKGANNSNT TNPPPAPSKS PEEMRKLFIQ RAKKIDKISR IGFPMAFLIF 420<br>NMFYWIIYKI VRREDVHNQ 439 |
| **3-98** | **Sequences** | |
| 3-98-1 | Sequence Number [ID] | 98 |
| 3-98-2 | Molecule Type | AA |
| 3-98-3 | Length | 439 |
| 3-98-4 | Features Location/ | **REGION 1..439** |
| | Qualifiers | note=synthetic sequence of chimeric LGIC |
| | | **source 1..439** |
| | | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-98-5 | Residues | MRCSPGGVWL ALAASLLHVS LQGEFQRKLY KELVKNYNPL ERPVANDSQP LTVYFSLSLL 60<br>QIMDVDEKNQ VLTTNIWLQM SWTDHYLQWN VSEYPGVKTV FFPDGQIWKP DILLYNSADE 120<br>RFDATFHTNV NVNSSGHCQY LPPGIFKSSC PMDLKNFPMD VQTCKLKFGS WSYGGWSLDL 180<br>QMQEADISGY IPNGEWDLVG IPGKRSERFY ECCKEPFPDV TFTVTMRRRM GYYLIQMYIP 240<br>SLLIVILSWI SFWINMDAAP ARVGLGITTV LTMTTQSSGS RASLPKVSYV KAIDIWMAVC 300<br>LLFVFSALLE YAAVNFVSRQ HKELLRFRRK RRHHKSPMLN LFQEDEAGEG RFNFSAYGMG 360<br>PACLQAKDGI SVKGANNSNT TNPPPAPSKS PEEMRKLFIQ RAKKIDKISR IGFPMAFLIF 420<br>NMFYWIIYKI VRREDVHNQ 439 |