Optical functionalization of human Class A Orphan G-Protein Coupled Receptors

A Thesis Presented to the Faculty of the Graduate School of IST Austria (Institute of Science and Technology), Klosterneuburg, Austria In Fulfillment of the Requirement for the Degree of Doctor in Philosophy (PhD) by

MAURIZIO MORRI

March 2016

Supervisor: Prof. Dr. **Harald Janovjak** IST Austria, Klosterneuburg, Austria

Committee member: Prof. Dr. **Carl-Philipp Heisenberg,** IST Austria, Klosterneuburg, Austria

Committee member: Prof. Dr. **Stefan Herlitze**, Ruhr-Universität Bochum, Bochum, Germany

Chair: Prof. Dr. **Michael Sixt**, IST Austria, Klosterneuburg, Austria

The dissertation of Maurizio Morri, titled "Optical functionalization of human Class A Orphan G-Protein Coupled Receptors", is approved by:

Supervisor: Prof. Dr. Harald Janovjak, 1ST Austria, Klosterneuburg, Austria

Signature: 

Committee Member: Prof. Dr. Stefan Herlitze, Ruhr-Universitat Bochum, Bochum, Germany

Signature: 

Committee Member: Prof. Dr. Carl-Philipp Heisenberg, 1ST Austria, Klosterneuburg, Austria

Signature: 

Defense Chair: Prof. Dr. Michael Sixt, 1ST Austria, Klosterneuburg, Austria

Signature: 

©Maurizio Morri, July 2016

All Rights Reserved

I hereby declare that this thesis is my own work and that it does not contain other people's work without this being so stated; this thesis does not contain my previous work without this being stated, and the bibliography contains all the literature that I used in writing the dissertation.

I declare that this is a true copy of my thesis, including any final revisions, as approved by my thesis committee, and that this thesis has not been submitted for a higher degree to any other university or institution.

I certify that any republication of materials presented in this thesis has been approved by the relevant publishers and co-authors.

Signature: 

Maurizio Morri

July, 2016

1-Introduction

1.1 Definition of G-Protein coupled receptor

Membrane receptors share the property of being able to respond to an extracellular stimulus(i) and translate it in a large variety of intracellular signaling cascades that modify cell behavior. Membrane receptors can be divided in three principal groups:

- Ion channels that allow flow of ions across the cell membrane in order to maintain or alter the membrane potential.
- Enzyme-coupled receptors that act as enzyme themselves or coupled to intracellular enzymes.
- G-Protein coupled receptors that couple to membrane bound heterotrimeric GTP binding G-proteins (G-Proteins) regulating a series of heterogeneous intracellular signals.

Among membrane receptors, G-Protein coupled receptors (GPCRs) form the largest protein family in the mammalian genome. However, an exact size of this protein family has yet to be determined[1].
According to the classical definition of GPCRs, a membrane protein to be classified as GPCR has to show the following two characteristics[2]:

- Seven domains in the amino acid sequences formed each by 25 to 35 consecutive residues with high hydrophobicity.
- The ability to couple to one or several G-Proteins

The presence of the seven consecutive highly hydrophobic stretches of amino acid residues leads to the formation in the tertiary protein structure of a strongly preserved topology. Seven α-helices cross the cell membrane in an anti-

clockwise manner leading to the N-terminus being exposed to the extracellular side and the C-terminus exposed to the cell intracellular environment. Three extracellular and three intracellular loops connect the transmembrane domains. The overall structure can vary in length among the wide group of GPCRs, with the shorter receptors being around 300 amino acids long and the longest ones around ~1000 amino acids in length[3].

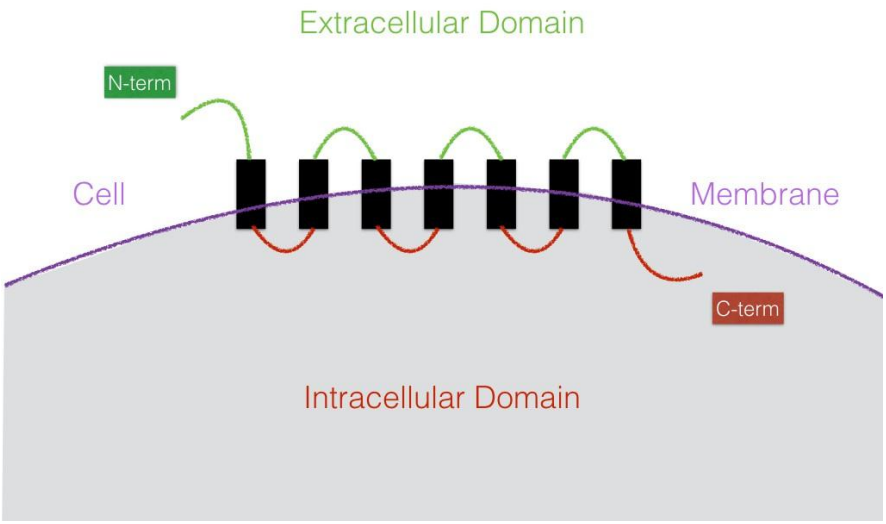

Figure 1.1 Topology of GPCRs: the seven transmembrane α-helices (black) connected by three intracellular loops (red, plus C-terminus) and three extracellular loops (green, plus N-terminus).

The GPCRs main function is to translate extracellular stimuli presented in form of a large variety of ligands into activation of intracellular signaling cascades. In the canonical description of these receptors the translation is achieved by activation of the heterotrimeric G-Proteins, but also a large variety of other proteins such as ß- arrestins and neurochondrin.

The activation of the G-Proteins has not yet been shown for most of GPCRs, especially for the ones recently discovered or predicted by genome analysis. This is the reason why it has been suggested to define GPCRs as seven transmembrane domain receptors[4], considering that the presence of the seven α-helices is always conserved, but the term GPCRs is nowadays still more widely established.

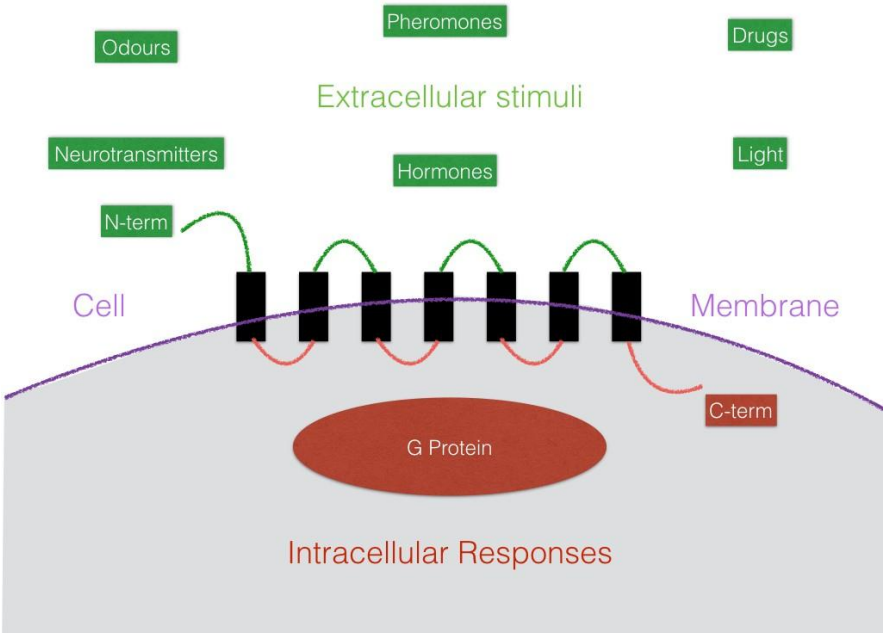

Figure 1.2 Different possible ligands that can interact with GPCRs and drive intracellular responses through activation of G-Proteins.

1.2 GPCRs classification

The ability of GPCRs to sense a wide range of extracellular inputs and drive different intracellular signal cascades is reflected in their heterogeneous sequences. Sequence similarity can however still be used as criteria to classify this large number of receptors in different subfamilies. Two classifications are currently used:

- The first classification divides GPCRs in different classes (from A to F) and clans (identified by a roman numeration) and it was proposed and introduced by both Atwood and Findlay[5], in separate work by Kolakowsky[6] in 1994 and refined by Bockaert and Pin[7] in 1999. This classification system spans all GPCRs in vertebrates and invertebrates, and it is based on the development of sequence-based fingerprints in the seven hydrophobic transmembrane domains common among GPCRs.

- A more complete view of the human repertoire of GPCRs was achieved in 2001 when the sequencing of the entire human genome was completed. This led in the following years to the development of an alternative

classification system[8]. This classification system called GRAFS has been proposed by Fredriksson[9] and divides the GPCRs expressed in the human genome in five main families: glutamate, rhodopsin, adhesion, frizzled/taste2 and secreting. The GRAFS system also provided a classification for the large family of rhodopsin like GPCRs.

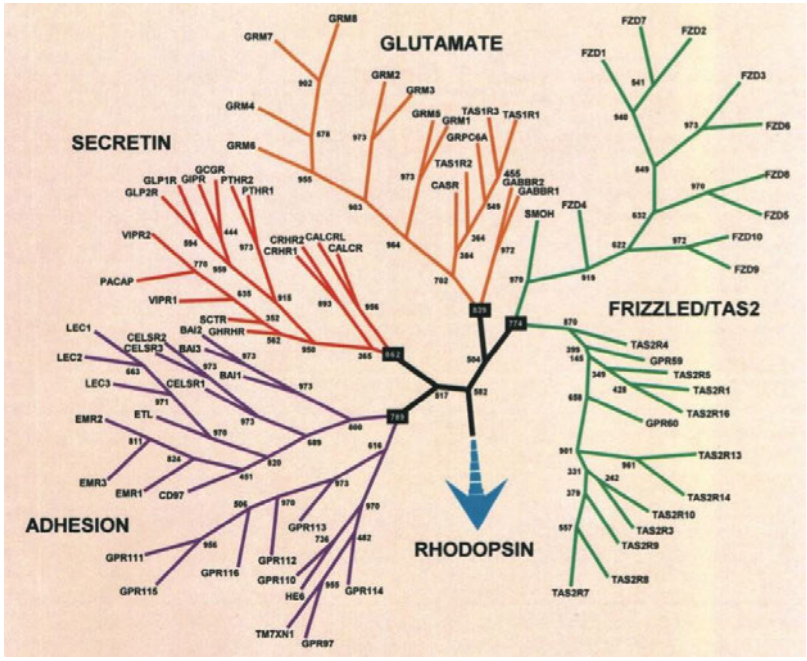

Figure 1.3 GRAFS classification of human GPCR genes based on their sequence homology according to Fredriksson *et al.*[9]

The main difference between the GRAFS classification systems and the formerly proposed system is the further division of the Class B GPCRs into secretin-like and adhesion GPCRs.

1.2.1 Class A (rhodopsin-like)

Class A GPCRs are the biggest and the most studied subfamily. The receptors belonging to this subfamily are also identified as rhodopsin-like receptors. All Class A GPCRs shared highly conserved common motifs with the amino acid

sequence of rhodopsin, although the overall homology across the entire subfamily can be low[10]. The common amino acid motifs are located in the transmembrane domains and in the first and second intracellular loops.
According to the different mechanism they used for the ligand binding process, Class A GPCRs can be divided in three different subgroups[11]:

- **A-α** that includes rhodopsin, adrenergic and dopamine receptors. The ligand binding mechanism in this receptor group is manly based on a core structure formed by the transmembrane domains.

- **A-β**, in which the ligand binding process is mostly carried out by the N-terminus plus extracellular loops as well as the transmembrane domains (ligands for this group are usually small peptides). Chemokines, opioids and somatostain receptors belong to this subgroup.

- **A-γ**, in which the ligand binding process is mostly carried out by the N-terminus of the receptor that in this group is longer in comparison with the previous two. Glycoprotein hormones such as luteinizing hormone belong to this subgroup.

- **A-δ**, in which the ligand binding process is mostly carried out by the N-terminus. Belongs to this group purinoreceptors 2 (P2RYs), glycol- protein binding receptors, protease-activated receptors (PARs) and olfactory receptors.

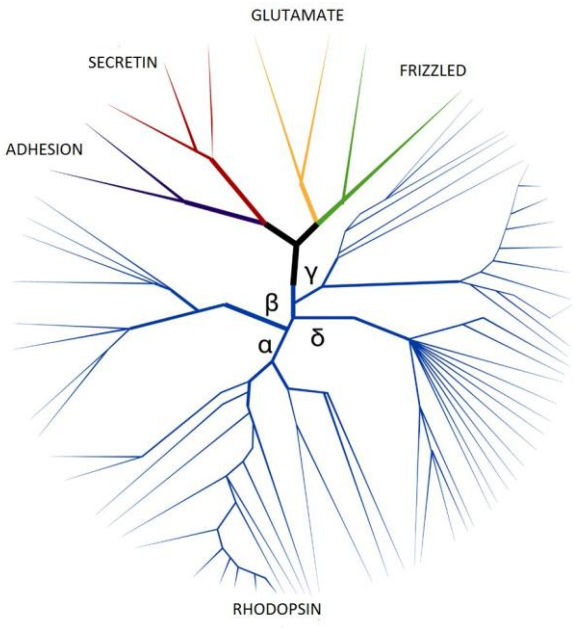

Figure 1.4 Classification of human Class A (rhodopsin-like) GPCR genes according to their different ligand-binding processes (adapted from Cardoso *et al.*[12])

1.2.2 Class B (secretin-like)

Class B GPCRs do not share most of the common motifs of the Class A GPCRs and additionally harbor a large extracellular domain that is involved mainly in the ligand binding process. The receptors that belong to this family are also known as secretin-like receptors. They are regulated by peptides belonging to the glucagon hormone family, involved in regulating important endocrine and neuroendocrine functions.

1.2.3 Class C (glutamate receptor-like)

Class C GPCRs, or the metabotropic glutamate/pheromone-like receptors, are characterized by an extremely long N-terminus (about 500 to 600 amino acids) and share a large hydrophilic extracellular agonist-binding pocket. These residues form a disulphide bond (the crystal structure for this ligand binding domain have been solved for metabolic glutamate receptors). This GPCR subfamily includes mostly glutamate receptors, as well as calcium and GABA receptors.

1.2.4 Adhesion GPCRs

Adhesion GCPRs, partially belonging to the class B GPCRs, are a set of ~30 receptors that present a hybrid structure. The peculiarity of their protein structure is an extremely long extracellular domain that includes different subdomains[13], responsible for facilitating and integrating the interaction between two different cells and between the cell and its surrounding matrix. The huge extracellular domain is connected to the seven-transmembrane domain in the cell membrane by a GAIN domain (GPCR-Autoproteolisis Inducing), responsible for the expression of the receptor at the cell membrane. Despite the main propose function for this receptor is probably to regulate cell position in space, it has been proposed an active role also for cell-cell communication[14] and cell migration[15], as well as in formation and development of cancer metastasis[16].

1.2.5 Other GPCRs classes

The remaining classes are formed by genes encoding for fungi GPCRs (**class D**) pheromones receptors (**Class E**), frizzled/smoothened receptors (**class F**) and a group of cAMP-receptors found in Dictyostelium discoideum (**class G**). Frizzled receptors present a common long N-terminus that is supposed to interact actively in the ligand binding process by exposing the binding pocket in the extracellular domain of the receptor normally masked. These receptors have a predominant role in embryonic development and present similarity with the class B GPCRs.

1.3 G-Protein classification

1.3.1 Common G-Protein activation mechanism

Once activated, GPCRs undergo conformational changes that trigger the activation of internal signaling cascades[17]. These internal signaling cascades are mediated by the interaction of GPCRs with the G- Protein gene ensemble. G-Proteins can be classified according to the secondary messenger they activate; however, a common activation mechanism for these proteins can be identified. Every G-protein should is composed by three different subunits, which

are defined as the α, ß and γ subunit. In the inactive state the α and the ß subunits are anchored to the plasma membrane by short lipid tails while the ß subunit is placed in between the α and the γ subunits, forming a tight complex[18]. The G-protein α subunit contains a binding site for the guanosine 5' diphosphate (GDP) that makes the G-protein idle in the inactive state. The activation of a GPCR act as guanine nucleotide exchange factor: the affinity of the α subunit for the GDP is reduced, leading to the release of the GDP from its binding site and causing its replacement by guanine 5' triphosphate (GTP) that is present in a higher concentration in the intracellular cell matrix. The dissociation of GDP from the α subunit causes the dissociation of the G-protein into two separate complexes: one of which is made by the α subunit and one formed by the ß and γ subunit tighten together. In this state, both the α and the ß-γ subunits are able to trigger different intracellular signaling pathways, translating the activation of the GPCR into a cellular response.

G-proteins can come back to their inactive state by its intrinsic GTPase activity that hydrolases the terminal GTP-phosphate. This brings back the α subunit to its inactive state in which it is bound to GDP and promotes the binding between the α subunit and the ß-γ complex.

The GTP-GDP exchange completed by the GTPase activity of the α subunit is not the only mechanism that revert to its inactive state the G-protein. Concurrent mechanisms have been proposed that involve regulators of G- protein signaling, or RGS proteins[19,20] (GTPase activating proteins). The RGS proteins have been proved to interact with the α subunit-GTP complex and promote the hydrolysis of the GTP in a shorter time scale in comparison with the one associated to the intrinsic α subunit GTPase activity.

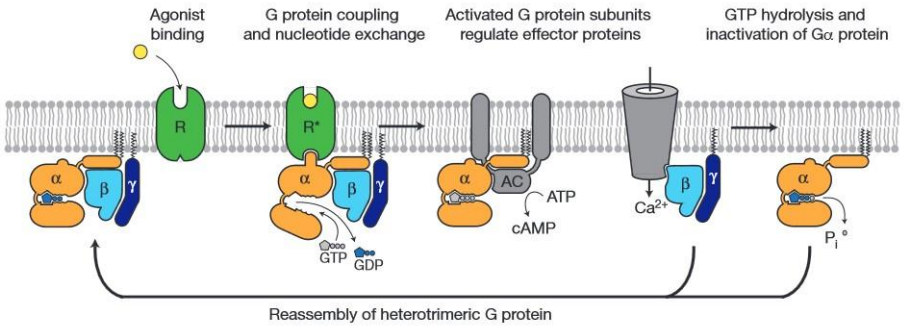

Figure 1.5 G-Protein activation cycle upon agonist binding: once the agonist binds to a GPCR, the GTP-GDP exchange enables morphological change and dissociation of the α and βγ subunits and the initiation of the signaling cascade (in the case in figure, cAMP production). GTP hydrolysis brings back the heterotrimeric G-protein to the initial inactive condition[21]

The previously described mechanisms control the length of activation for a G-proteins, depending on the action of the intrinsic GTPase activity of the α subunit and the RGS activity. In some specific cases the activated signaling cascade has an intrinsic GTPase activity, like phospholipase C-ß in its C- terminus[22,23] .

1.3.2 G-Protein subunits and their signaling functions

Sixteen different genes are currently identified for coding for 28 different G-Protein α subunits. These 28 different α subunits can be grouped into four families according to the second messenger they activate:

- **Gα-s** subunits activate production of cyclic AMP (cAMP) through adenylyl cyclase. It has been shown that Gα-s subunits can interact with potassium channels, Src tyrosine kinases and tubulin GTPase[24,26]. In the Gα-s family we list the Gα-s (S), the Gα-s (L) and the Gα-s (XL), s p l i c e d products of the same gene (*GNAS*). A forth Gα-s subunit is expressed mainly in olfactory and some CNS ganglia and called Gα-s-olf (*GNAL*).

- **Gα-i/o** subunits inhibit the production of cAMP by adenylyl cyclase and can be seen as having an inverse Gα-s physiological function. They can also activate potassium channels, inhibit calcium channels, activate RAP1GAPII-dependent ERK/MAP kinase and activate Src tyrosine kinases. There are five different versions of Gα-i/o:

- Gα-o1 (*GNAO1*) Gα-o2 (*GNAO2*), specifically expressed in neurons and neuroendocrine cells[27,28].
- Gα-i1-3 (*GNAI1, GNAI3*), ubiquitously express although it has been found preferential expression in neuronal system[29,30].
- Gα-z (*GNAZ*), found predominantly in neuronal tissues, which has functional roles remained largely undefined[31].
- Gα-t1/2 (*GNAT1, GNAT2*) stimulate cGMP phosphodiesterases and have a highly specific expression in the visual system (retinal rode and cone outer segments) although expression has been reporter also in taste buds[32,33].
- Gα-gust (*GNAT3*) has a specific taste function and it has been reported express in the taste buds involved in the sweet and bitter taste and also in chemoreceptors in the airways[34].

- **Gα-q/11** subunits have all the common function of activating phospholipase Cbetha (PLCbetha) isoforms. PCLbetha in the active form hydrolase the phospholipid phosphatidylinositol 4,5 biphosphate to 1,2 diacyclycerol (DAG) and inositol 1,4,5 triphosphate (IP3). IP3 interact with specific IP3 channel that are expressed in the smooth endoplasmatic reticulum (SER)[35]. The release of calcium from IP3 channels establishes a positive feedback that drives the release of more calcium from other IP3 channels. Ryanodine receptors can also be activated by this positive feedback system, increasing the release of calcium. The initial concentration of calcium in the intracellular matrix is restored by the activation of calcium ATPases on the plasma membrane and endoplasmatic reticulum and by calcium and sodium channels. Five different α subunits belong to the Gα-q/11 family: Gα-q (*GNAQ*), Gα-11 (*GNA11*), Gα-14 (*GNA14*), Gα-15 (*GNA15*) and Gα-16 (*GNA16*). Gα-q

and Gα-11 are widely expressed, while the rest of the members of the family are specifically expressed in hematopoietic cells. Also, one striking difference is that Gα-11, Gα-14, Gα-15 and Gα-16 are known to be promiscuous Gα subunits involved in the coupling of Gα-s and Gα-i/o coupled GPCRs with the IP3 pathway[36-38].

- **Gα-12/13** *(GNA12, GNA13)* are widely expressed and are associated with responses linked to monomeric GTP-binding G-Proteins such as RAS. When RAS is bound to GTP and activated, it starts a phosphorylation cascade that end with the activation of mitogen- activated protein kinases MAPK. The activation of MAPK leads to the phosphorylation of various effector proteins that effect gene transcription and influence cell behaviors such as cell proliferation, differentiation and morphology[39,40].

1.4 GPCRs structure

1.4.1 Data gathering for GPCR crystal structures

Only 148 GPCRs solved crystal structures belonging to 23 different GPCRs are currently published.

Receptor name	Receptor ID	PDB ref. num.	GPCR class
Muscarinic Receptor 2[41,42]	CHRM2	4MQT, 4MQS, 3UON	Rhodopsin-α
Muscarinic Receptor 3[43,44]	CHRM3	4DAJ, 4U14,4U15, 4U16	Rhodopsin-α
Dopamine Receptor 3[45]	DRD3	3PBL	Rhodopsin-α
Serotonin 5-HT1b Receptor[46]	HTR1B	4IAQ, 4IAR	Rhodopsin-α
Serotonin 5-HT2b Receptor[47, 48]	HTR2B	41B4, 4NC3	Rhodopsin-α
β2 Adrenergic Receptor[21]	ADRB2	4QKX, 4LDE, 4LDL, 4LDO, 4GBR, 3SN6, 3P0G, 3PDS, 3NY8, 3NY9, 3NYA, 3KJ6, 3D4S, 2R4R, 2R4S, 2RH1	Rhodopsin-α
β1 Adrenergic Receptor[49]	ADRB1	1DEP, 2VT4, 2Y00, 2Y02, 2Y03, 2Y04, 2Y01, 2YCW, 2YCX, 2YCZ, 2YCY, 4AMI, 4AMJ, 4GPO, 3ZPQ, 3ZPR, 2LSQ, 4BVN, 5A8E, 5F8U	Rhodopsin-α
Rhodopsin[50]	RHO	4ZWJ, 4WW3, 4BEY, 4BEZ, 4A4M, 3AYM, 3AYN, 2X72, 3PQR, 3PXO, 3OAX, 3C9L,	Rhodopsin-α

		3C9M, 3CAP, 2Z73, 2ZIY, 2PED, 2J4Y, 2J28, 2I35, 2I36, 2I37, 2G87, 2HPY, 1U19, 1GZM, 1LN6, 1L9H, 1JFP, 1HZX, 1F88	
Histamine 1 Receptor[51]	HRH1	3RZE	Rhodopsin-α
A2A Adenosine Receptor[52,53]	ADORA2A	4UG2, 4UHR, 3UZA, 3UZC, 3VG9, 3VGA, 3PWH, 3REY, 3RFM, 2YDO, 2YDV, 3QAK, 3EML	Rhodopsin-α
Purinergic P2Y12[54,55]	P2Y12	4PXZ, 4PY0, 4NTJ	Rhodopsin-δ
Protease-activated Receptor[56]	PAR1	3VW7	Rhodopsin-δ
Neurotensin Receptor 1[57]	NTSR1	3ZEV, 4BUO, 4BV0, 4BWB, 4XEE, 4XES, 4GRV	Adhesion
Corticotropin releasing factor 1 Receptor[58]	CRHR1	3EHT, 3EHU, 2L27, 4K5Y	Secretin
Glucagon Receptor[59]	GCGR	2A83, 3CZF, 4ERS, 4L6R, 4LF3, 4L6R	Secretin
Smoothened Receptor[60]	SMOH	4N4W, 4JKV , 4N4W	Glutamate
Glutamate mGluR1 Receptor[61]	GRM1	1EWK, 1EWT, 1EWV, 1ISR, 1ISS, 3KS9, 4OR2	Glutamate
CXCR4 Chemokine Receptor[62]	CXCR4	4RWS, 3ODU, 3OE0, 3OE6, 3OE8, 3OE9, 2K01, 2K03, 2K04, 2K05, 1VMC, 2SDF, 1SDF	Rhodopsin-γ
C_C Chemokine Receptor 5[63]	CCR5	4S2S, 4MBS, 2QAD	Rhodopsin-γ
Nociceptin Receptor[64]	ORL1	5DHG, 5DHH, 4EA3	Rhodopsin-γ
Kappa-Opioid Receptor[65]	OPRK1	4DJH	Rhodopsin-γ
Opioid Receptor, Mu 1[66]	OPRM1	4DKL, 5C1M	Rhodopsin-γ
Opioid Receptor delta 1[67]	OPRD1	4EJ4, 4N6H, 4RWA, 4RWD	Rhodopsin-γ

Table 1.1 List of available 3D GPCR crystal structures solved by X-Ray diffraction and laser-based imaging techniques with relative PDB reference numbers

In most cases the GPCR is crystalized together with an agonist or antagonist that has the function to stabilize the protein crystal, making the structure acquisition easier. Only in two cases was it possible to acquire a diffraction pattern from a GPCRs bound to the related G- Protein complex[21,68].

The surprisingly low amount of crystal structures can be explained by taking in account that the protein crystallization process is not trivial, especially in the case of proteins like GPCRs. The presence of loops on the intracellular and the extracellular side and the elasticity suggested by morphological changes upon activation makes even more difficult to obtain a sufficiently resolved diffraction spectra that allow to identify a crystal structure[69,70].

The use of Synchrotron Radiation sources as well as improved crystallization protocols might increase the limited number of solved 3D GCPR crystal structures[71]. However, taking in account the topological resemblance between GPCRs, it is possible to use solved crystal structures to model mechanism of ligand binding, signal transduction and morphology changes as well as other common protein mechanisms.

1.4.2 Current GPCR model based on crystal structures

The crystal structure of bovine rhodopsin is considered as a good model for describing GPCRs. Indeed, the majority of the GPCRs are identified as belonging to the Class A subfamily. Second, the intermediate amino acid sequence length of rhodopsin among the GPCRs suggests that the functionally important sequences are included in the rhodopsin sequence, while the poorly conserved sequences are most likely located at protein sites responsible for the interaction with the G-protein (i.e. the intracellular loops or at the N-terminus level).

The first crystal structure of the inactive state of bovine rhodopsin was the first GPCRs crystal structure solved by Palczewski *et al.* [50] in 2000. Upon the absorption of a photon, the 11-cis retinal linked to the Lys296 of the rhodopsin sequences undergoes an isomerization in all-trans state, leading to a conformational change of the protein structure. Hydrolization of the all-trans retinal leads to retinal dissociation from rhodopsin that can be regenerated by new 11-cis retinal produced by retinoid isomerohydrolase (RPE65) in retinal epithelial cells. The conformational changes upon light activation can be performed by rhodopsin thanks to the flexibility of its transmembrane domains (between 19 and 34 amino acids long and able to be tilted and kinked).

Different features of the rhodopsin crystal structure are found to be conserved in many other GPCRs, not exclusively belonging to the Class A. An Asp/Glu-Arg-Tyr (D/ERY) motif is found at the end of the TM3 and well conserved among

most Class A GPCRs. It is involved in receptor stabilization in the inactive state and in the activation process itself. In the TM7, a highly conserved NPXXY motif is present in order to give to the receptor structure stability by interaction with the adjacent TM6. The extracellular loops contain two cysteine residues highly conserved among all GPCR families. These residues are supposed to increase the receptor structure stability by forming a disulphide bond.

One attribute of the rhodopsin structure is the presence of the ionic lock, a salt bridge between the Arg residue in the (D/ERY) motif in the TM3 and a Glu residue in the TMVI[72]. The same Arg residue forms another salt bridge with the adjacent Asp residue that is found to be present in all other class A GPCRs in their inactive state.

The presence of the salt bridge between the Arg and the Glu in TM3 and TM6 is the reason why rhodopsin has no basal activity and a stable ON state: upon interaction with a photon, the isomerization of the 11-cis retinal and consequent structural changes allows the break of the salt bridge that is not present in any active state of rhodopsin[73].

The break of the ionic lock allows the interaction of the TM3 and TM6 with the G-Protein in the receptor active state. Mutations in the amino acid residues involved in the formation of the ionic lock lead to modifications in the constitute and agonist-binding activity.

In 2011, Standfuss *et al.*[74] solved the crystal structure of a mutated-constitutively active form of bovine rhodopsin, together with a peptide derived from the C-Terminus of the α-subunit of the Gα-t1/2. This crystal structure suggests that the rotation of the TM6 is critical in the conformational changes involved in rhodopsin activation, and also how the rearrangement of hydrogen bonds between the retinal-binding pocket and the previously cited common GPCRs motif can be proposed as common mechanism of agonist-related GPCRs activation. In 2011 the crystal structure of the complex between the ß2- adrenergic receptor (B2AR) and the Gα-s subunit was solved by Rasmussen *et*

al.[21] This was the first crystal structure ever showing a high-resolution view of transmembrane signaling by GPCRs.

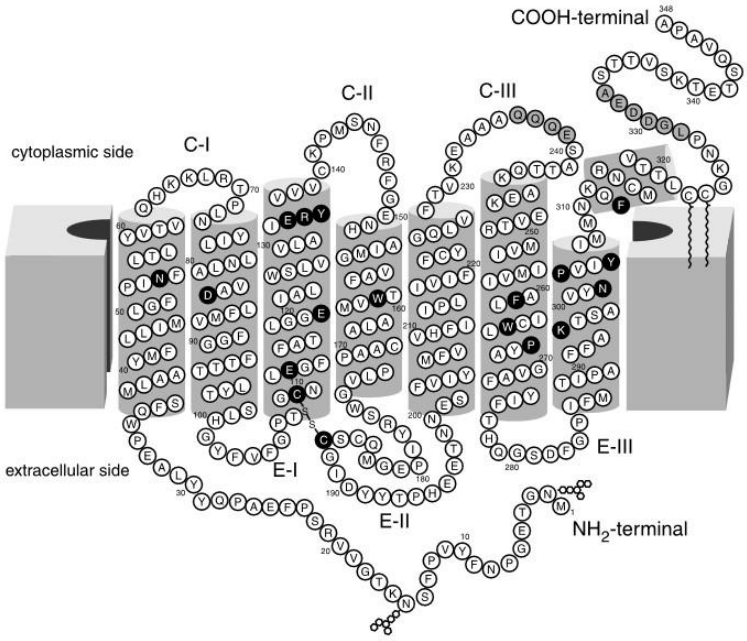

Figure 1.6 Rhodopsin amino acid sequence represented in a structure-based model (from top to bottom: intracellular loops [C-I, C-II, C-III and COOH-terminal], transmembrane domains and extracellular loops [E-I, E-II, E-III and NH_2-terminal] sequences)[50]

The additional information brought by this crystal structure describe the interaction between the B2AR and the Gα-s protein as mainly focused on the C-terminal α helices of the Gα-s protein and the nucleotide-binding pocket. Also, in the active state the B2AR shown a displacement of the TM6 of ~14 Å caused by the interaction with the Gα-s protein.

1.5 Mechanisms of GPCR activation

1.5.1 A two states model of GPCR activity

In a simple description, a GPCR can occupy two different energetically stable states: a ground state (or inactive state) and an “excited” state (or active state), in which receptor activation and corresponding intracellular signal transduction are started by the ligand-receptor interaction[75]. This description is missing

important aspect of GPCRs activity such as constitutive activity[76] and intermediate states of activation, but it gives a general idea of the different mechanism of interaction between a GPCR and its ligands[77].

Rhodopsin and the ß2-adrenergic receptor with their corresponding crystal structures can be consider as standard reference model to explain ligand- binding mechanism for GPCRs[78,79]. Also, mutagenesis studies in biogenic amine receptors such as the α1 and ß2 adrenergic receptors, the H2 histamine receptor and the muscarinic acetylcholine receptor family show peculiar binding sites and morphological changes in the ligand binding process.

In rhodopsin, 11-cis retinal acts as an inverse agonist, keeping the receptor inactivated in the dark. The 11-cis retinal is covalently bond to Lys296 in the TM7. The exposures to light cause the isomerization of the 11-cis retinal in all- trans retinal that has an agonist function in rhodopsin activation.

In the cases of ligand-activated GPCRs, such as the bioamine receptors (Class A GPCRs), the ligand-binding pocket is formed by different transmembrane domains, where the main interaction happens at TM3, TM5 and TM6. It has been shown by using mutagenesis approaches that the interaction between the positively charged amine and the receptor is mainly controlled by a highly conserved aspartic acid residue in the TM3.

This mechanism of activation that involves the interaction between the ligand and the transmembrane domains of GPCRs shown in rhodopsin and bioamine receptors is actually not common in all Class A GPCRs[80,81].

In the case of peptide activated GPCRs (Class A), such as angiotensin and chemokine receptors, the mechanism of ligand binding involves also the N-terminus and the first and second extracellular loops. The transmembrane domains are also involved, in particular the TM6 and TM7[82].

For receptors such as adenosine receptors (Class A) the ligand binding process more close resembles the rhodopsin-ß2-adrenergic model, with an important role of the transmembrane domains together with the second extracellular loop.

Among Class A GPCRs, protease-activated receptors present a unique mechanism of activation. They have on the N-terminus a site for cleavage by protease such as thrombin. Once the receptor's N-terminus is cleaved, this act as agonist by interacting with its own extracellular loops, in a way comparable to the peptide activated GPCRs[83-85].

For the other GCPRs classes previously described, there is a prominent role of the N-terminus in comparison with the Class A GPCRs. In the case of Class C metabotropic glutamate receptors, the complete ligand-binding site is made by the receptor N-terminus[86]. As shown in the metabotropic receptor 1 (mGluR1, Class C GPCRs) crystal structure, the formation of a disulphide-linked dimer allows an open-close formation in response to ligand binding[87].

The two state model is actually a very good approximation in the case of rhodopsin, in which the presence of the 11-cis retinal as inverse agonist brings close to zero the probability of receptor activation in the dark. Retinal isomerization (and consequently the presence of light) is necessary condition to allow the conformational changes necessary to induce signal transduction by rhodopsin. For all other GPCRs, including genes belonging to the Class A rhodopsin-like, it has been recorded widely spontaneous activation in the absence of any agonist, and this phenomenon is defined as constitutive activity.

1.5.2 Beyond the two state model

The two state model is consistent with GPCRs activation curves obtained from radio ligand-binding assay and it is able to explain a variety of effects, such as basal activity and ligand potency of adrenergic and muscarinic receptors. Also, constitutive activity can be explained using this model with statistical arguments: without the presence of activating molecules, a GPCR population would be mostly found in its ground or inactive state (G1). If the difference in energy between the ground state and the excited (activated, E1) state is low enough, it will be possible to observe spontaneous transitions

between the G1 and E1, also without the presence of an agonist. In this way the constitute activity of GPCRs can be described as a product of statistical oscillations in the receptor population, depending on the difference in energy levels between the ground and active states at equilibrium. The presence of an agonist modifies this equilibrium, moving the GPCR statistical distribution towards a majority of receptor being in the E1 state instead of the G1.

Experiments such as fluorescence quenching studies on amine receptors suggest the presence of different agonist induced receptors conformation rather than an agonist dependent action in modulating the equilibrium between a single inactive and active state[88]. The activity of GPCRs might be determined by a series of structural switches upon activation and different agonist might have different abilities to modulate the activity of these switches.

These recent results made necessary to modify also the simple model based on agonist-antagonist effects when talking about GPCR-ligand interactions. The classical definition of agonist has to be updated: a molecule can be defined as a full agonist when the interaction between the molecule and the GPCRs produces a maximal effect (i.e. signal intensity) in a specific assay. A partial agonist is a molecule that produces a signal detectable, submaximal and bigger than the constitute activity one. GPCRs antagonist can be defined as molecules that block the interaction between the agonist and the GPCRs, but also as compounds that blocks the receptor state at the ground state G1, removing constitutive activity. The efficiency of this blocking action makes possible to distinguishes between molecules that are full antagonists and partial antagonists.

In the case of rhodopsin, the active state E1 is attained through the formation of multiple intermediate states. The G1 state (or dark state) is maintained by the action of the 11-cis retinal. Without the 11-cis retinal rhodopsin shows a basal activity a million time smaller than the one registered in the fully activated E1 state. The spectral property of the 11-cis retinal allow to monitor the multiple intermediate states that can be trapped at low temperature and can be detected at physiological temperatures in two-dimensional crystals[89].

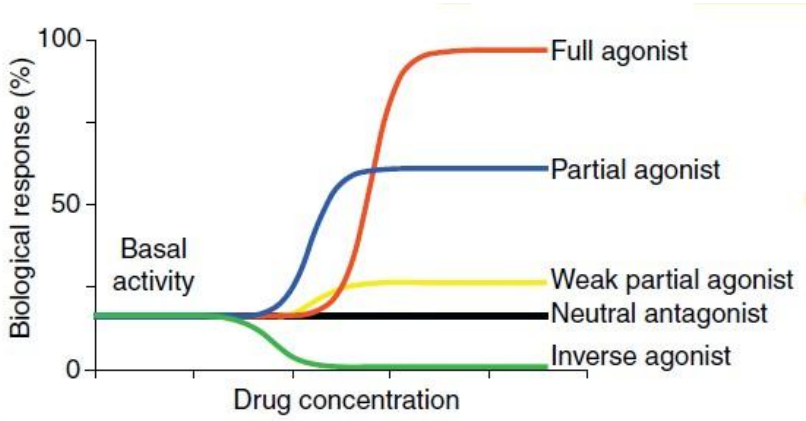

Figure 1.7 Different pharmacological roles of GPCR ligands represented as potency of activating or inhibiting biological responses.

The structures of the dark ground state of rhodopsin show high similarity with respect to the transmembrane domains, while they differ the most at the level of the cytoplasmic loops[90] (due also to the flexibility of the loops themselves).

For three of the inactive intermediate states of rhodopsin crystal structures are also available. Bathorhodopsin and lumihodopsin are basically isomorphus of the dark state receptor structure. Although the isomerization process that lead to the conformational change of retinal from 11-cis to all trans is completed by the stage of the lumirhodopsin, this results only in minor local changes in the structure of the receptor.

It has been observed that GPCRs can couple to different G-Proteins according to the full or partial agonist that activates them. Different agonists can promote distinct conformational states that dictate the class of G-protein to couple or if a G-Protein independent pathway will be activated.

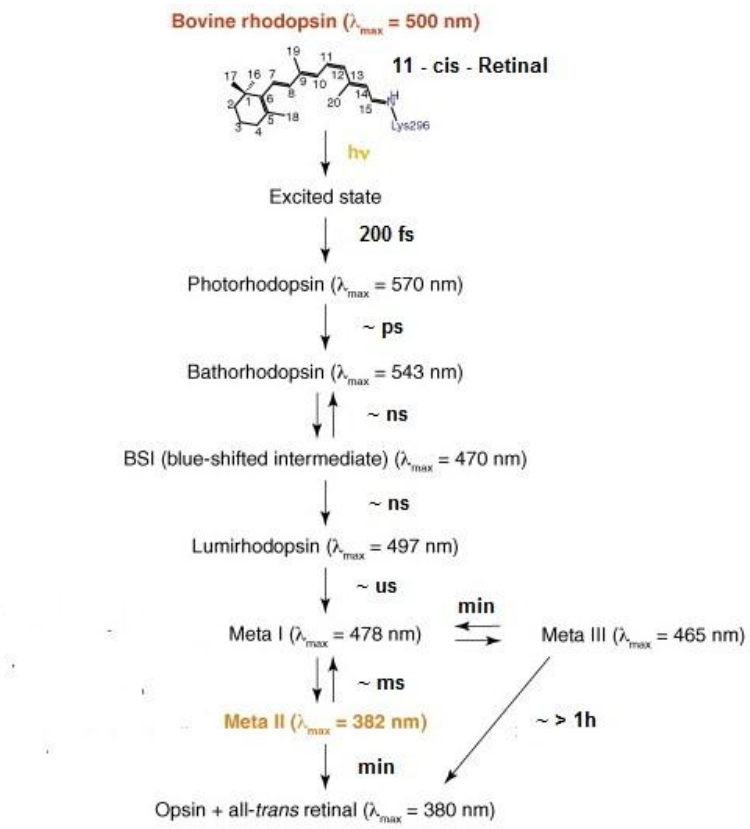

Figure 1.8 Rhodopsin activation mechanism through different intermediated meta-states with relative absorption spectra maxima and estimated life-times

Most of the available information about GPCR structures comes from X-ray spectroscopy techniques. One of the limit of this approach is that it gives access to structural information only of GPCR states that are stable enough to be crystalized with sufficient purity to get a readable diffraction spectrum. This limit makes not accessible to X-ray spectroscopy many other less stable states that could have however important functional roles

This notion that different active conformational states give GPCRs the ability to stimulate a diverse array of signaling pathways is defined as ligand bias or functional selectivity[91]. Actually, ligand bias and functional selectivity for GPCR are two slightly different concepts[92]:

- Ligand bias is a system-independent concept. It reflects how different ligands might thermodynamically control through energy exchange stabilization of distinct conformations of the ligand-bound receptor, exposing different receptor coupling mechanisms. This implies that although in vivo signaling assays might be influenced by presence and distribution of receptor coupling properties, in vitro assays can identify intrinsic bias associated with specific ligand-receptor states.

- Functional selectivity is general concept that describes differential pharmacological effects of ligands on specific GPCRs including pharmacokinetics, target receptor conformation and activation of different molecular targets. Unlikely ligand bias, functional selectivity is system dependent and it is indeed the most appropriate term to use when speaking about in vivo pharmacology tested on different assays in different systems.

The main consequences of GPCRs functional selectivity is that the pharmacological properties and classification of ligands (full or partial agonist, antagonist etc.) will depend upon their efficacy, target and biochemical pathways investigated[93,94].

The search for ligands having the properties of modulating different responses of a specific target receptor has pushed the development of techniques for large compound screening. While such techniques are currently available for screening G-protein mediated pathways, in the last years a significant number of techniques have been developed to screen for ß-arrestins activation for GPCRs. One of the earliest techniques to monitor ß-arrestins-GPCR interaction was based on the use of a fluorescent-tagged ß-arrestin and the study of its relocalization upon GPCR activation (transfluor approach). A more recent approach is based on bioluminescent energy transfer (BRET): both ß-arrestins and the target GPCR are tagged with a fluorescent protein and Renilla luciferase (the GPCR is tagged on its C-terminus). When the two proteins get close enough, the bioluminescence emitted from the Renilla luciferase is enough to excited the fluorescent tag that will then emit a detectable signal at higher wavelength.

A similar approach, also based on proximity between GPCRs and ß-arrestins, is the so-called TANGO approach[95, 96]. The target GPCR gets tagged at the C-terminus with a transcription factor (Gal4) using a protease cleavage site.

The ß-arrestin is tethered with a Tobacco Etch Virus nuclear-inclusion-an endopeptidase (TEV). When the two proteins are close enough, the protease on the ß-arrestin cleaves the linker site on the GPCR C-terminus, with consequent release of the transcription factor. The screening system expresses a ß-lactamase reporter gene, which is activated by the Gal4 transcription factor.

The previously described techniques were used to detect ligand bias properties for different GPCRs. Probably the most studied GPCR for ligand bias is the angiotensin II type 1 receptor[97] (AT1R). Ligands that stimulate ß-arrestin mediated pathways through AT1R are proved to have anti-apoptotic and cardio protective functions. Other GPCRs for which ligand bias (in vitro) and functional selectivity (in vivo) has been proved are H-HT4 and H-HT7 serotonin receptors[98,99], β1 and α2 adrenergic receptors[100,101], dopamine 2 receptor[102], histamine 2 and histamine 4 receptors[103-105], EDG1 receptor[106] and the glucagon-like peptide-1 receptor[107].

1.6 Orphan G-Protein coupled receptors (oGPCRs)

In the human genome around 350 genes encode for not-olfactory GPCRs, and for about 200 of them a candidate agonist has been determined. For the remaining genes, any agonists have no yet been discovered, making them orphan G-Protein coupled receptors[108] (oGPCRs). As for the other GPCRs, also the oGPCRs can be divided by sequence homology in different families, placing most of them in the Class A rhodopsin- like family. Considering the importance of GPCRs in drug development, different approaches have been used in order to identify possible agonists[109].

The first approach to be used was the “reverse pharmacology”: enormous libraries of different exogenous compounds were screened by monitoring the change in level of intracellular second messengers. Later on the reverse pharmacology approach was enhanced in the so called “orphan receptor strategy”, in order to increase the rate of deorphanization of o G P C R s [110].

The orphan receptor strategy’s aim is to reduce the amount of compounds included in the screening process by using only extract from tissue in which the oGPCR was found mostly expressed. The candidate (ant)agonist in this technique is isolated by further titration of the tissue extract and finally identified using mass spectroscopy techniques[111].

These two process focus on measuring the change in the intracellular level of second messenger upon interaction of the receptor with a possible agonist. Always looking at the intracellular environment, it is possible to detect oGPCR activation by monitoring the level of protein connected to the internalization of the receptor such as ß-arrestin2 (in a modified GFP tagged version). This approach, called “transfluor technology”, quantify the modification in distribution of ß-arrestin2 from broadly in the cell cytosol to a localized distribution as a consequence of ligand-dependent internalization[112]. oGPCRs can also be extracellular-tagged using a pH sensitive fluorescence antibody such as CypHer5. Cypher5 is a cyanine pH-sensitive dye not fluorescent at pH7.4 and brightly fluorescent at lower pH. Due to the acidic nature of the endosomal recycling compartments, the use of the cyanine dye Cypher5 permits to identify ligand-dependent internalization by measuring an intense intracellular fluorescence.

Despite the availability of different approaches, the rate of deorphanization of oGPCRs in the last ten years has decreased in a sensitive manner[113]. This might have different explanations. For example, oGPCRs might have a not G- Protein mediated signaling function not possible to detect with the current screening methods, or the lack of accessory proteins such as ß-arrestins in the screening essay might as well interfere with the expression, the ligand and signaling coupling properties of the oGPCRs[114].

1.6.1 Class A oGPCRs

oGPCRs are distributed among the different GPCRs subfamilies according to their sequence similarity with not-orphan GPCRs belonging to the same subfamily. In human, most oGPCRs belong to the Class A rhodopsin-like family. In April 2011 the IUPHAR (International Union of Basic and Clinical Pharmacology) database listed 94 genes supposed to be human Class A oGPCRs.

The composition of this family is heterogeneous, and it is possible to identify different subfamilies among the family of human Class A oGPCRs[115].

- **65** genes are considered as human Class A oGPCRs, but due to their lack of similarity with other GPCRs subfamilies they are currently identified with a GPRXX (where xx is a number) nomenclature.
- **Eight** genes are identified as MAS related GPCRs (MRGPRD, MRGPRE, MRGPRF, MRGPRG, MRGPRX1-X2-X3-X4).
- **Seven** genes are identified as trace amine associated receptor (TAAR2-3-4-5-6-8-9).
- **Three** genes are classified as leucine-rich repeat containing G-protein-coupled receptor (LGR4, LGR5, LGR6).
- **Two** genes are identified as proto-oncogenes (MAS1, MAS1L).
- **Two** genes are identified as opsins (OPN3, OPN5).
- **One** gene is identified as a lysophosphatidic acid receptor 6 (LPR6).
- **One** gene is identified as an oxoglutamate receptor (OXGR1).
- **One** gene is identified as a purinergic receptor (P2RY8).
- **One** gene is identified as a succinate receptor (SUCNR1).

Human Class A oGPCR present a wide range of expression[116,117]. Most of them are expressed ubiquitously in human tissues, while a considerable number of

oGPCR genes is found expressed in the brain and in the central nervous system (suggesting that these receptors might have a physiological relevance in neurological functions). Several other oGPCR genes are expressed in organs involved in the immune response such as bone marrow, thymus, spleen and lungs.

Another hint suggesting that human Class A oGPCRs might have relevant physiological roles is coming from observed phenotypes in case of knockout and overexpression in murine models of these receptor[118].

For class A oGPCR genes there are no or few information about possible candidate agonists and relative G-Protein mediated signaling pathways that they might activate. This lack of information has driven in the past years the rise of alternative-signaling hypothesis for oGPCRs.

1.6.2 Class C oGPCRs

Six oGPCRs have been group in the Class C GPCR subfamily. GPR5CA-B-C-D all shares a similar N-terminus structure, short and containing two conserved cysteine residues. These four oGPCRs can be divided into two clusters based on sequence analogy (limited to the transmembrane domains): one formed by GPRC5B and 5C that are 50% identical, and one formed by GPRC5A and 5D that are 52% identical. The sequence identity between these two clusters can be measured around 40%. Both clusters contain typical conserved motifs of Class C GPCRs (such as the W in the TM6 and the P in the PKXY motif in the TM7) that are involved in the activation machinery and G-Protein signaling. Two other orphans were identified as belonging to Class C GPCRs, GPR158 and GPR158L that don't show any conserved domain in their respective N termini. Only recently, GABABRL (a new proposed member of the GABABR group) was proposed to be added to the list of Class C oGPCRs. GABABRL shows a sequence similarity with GABABR1 and GABABR2 of ~30%, but cells that express GABABRL alone or co-localized with GABABR1 or GABABR2 were not able to respond to GABA, suggesting that GABABRL might be a GABABR-like orphan with the ligand still awaiting identification.

1.6.3 Adhesion oGPCRs

Most of adhesions GPCRs are considered as orphans, including LEC receptors and EGF-TM7-lathophilin related protein[119,120] (ETL/ELTD1). While the endogenous ligand for LEC receptors is still unknown, α-latroxin (a molecule present in the venom of the black widow spider) can bind the extracellular adhesion part and the first transmembrane domain with consequent activation of the LEC1 receptor.

Three cadherin EGF LAG seven-pass-G-Type-receptors (CELSR1-2-3) are contained in the human genome. Mutations in the last cadherin domain of mouse CELSR1 gave rise to the spin cycle mutant, inducing a phenotype with abnormal head-shaking behavior and neural tube effect.

GPR64, GPR97, GPR111 till 116, GPR123, GPR 126, GPR128, GPR133, GPR144, three brain angiogenesis inhibitor (BAI) and MASS1 are all considered as adhesion oGPCRs. In rat, GPR116 has been shown to exist as a homodimer that is linked by disulphide bonds and it has been shown that can undergo endoproteolytic cleavage.

The MASS1 can be expressed as three different isoforms with its longest isoform being composed of ~6000 amino acids. Mutations in the gene are associated with audiogenic seizures in mice.

1.7 Physiological relevance of oGPCRs in human diseases

Information about oGPCR role in physiology can currently only be obtained in case of constitutive activity and by knockout studies. Also, high expression of an oGPCRs in a specific tissue and/or specific environmental conditions (i.e. cancer, inflammation, etc.) can be interpreted as a role that oGPCR in that specific physiological context.

1.7.1 Tumorigenesis and metastatic formation

Orphan GPCRs are physiologically relevant in different kind of cancer, such as triple negative breast cancer[121], skin and lung cancer. In last years, attention

was focused on GPR161[122], which is expressed in the triple negative breast cancer. This cancer is known to be connected with a poor diagnosis, decreasing the possibility of success of the usual treatment approaches. The overexpression of GPR161 in this cancer increases cell proliferation and migration enhancing cancer metastatic activity[123,124]. In contrast, the knockout of the GPR161 gene impairs the proliferation of human breast cancer cell lines, making GPR161 a good candidate target for new therapeutic approaches. GPR19[125,126] is as well considered a possible target to treat small-cell lung cancers (SCLC or oat cell cancer, about 10% to 15% of all lung cancers), due to its overexpression in tissue sample of lung cancer patients. GPCR5a[127,128] in breast cancer and GPR34[129,130] in lymphoma are supposed play a relevant role in the metabolism and growth of the relevant cancer cells types.

1.7.2 Neurological and psychiatric disorders

Most of the human oGPCRs are expressed in different parts of the nervous system, especially in the brain. Several studies proposed that oGPCRs might have a physiological relevant role in neurodegenerative and psychiatric diseases, as well as high-order functional brain activities. Among all, GPR37 and GPR37L1 have attracted attention in the last years[131,132] because they are expressed exclusively in the brain (both in neurons and glia) and they are both associated with juvenile Parkinson's disease. Knockout of GPR37 in murine models leads to an underdevelopment of the dopaminergic tone in the brain and the arising of perturbation of dopaminergic signals.

GPR6[133,134] , GPR52[135,136] and GPR88[137,138,139] are in various forms connected to major mental illness by altering the dopaminergic system in the striatum. The knockout of GPR6 leads to reduction of production of cAMP in the striatopallidal neurons and consequent alteration of the striatal dopaminergic system. GPR6 knockdown mice show reduced abnormal involuntary movements. In mice missing GPR52 a psychosis-related behavior was recorded, while GPR52 transgenic mice show an opposite antipsychotic behave. GPR88 is highly expressed in Dopamine1 (D1R)-Dopamine2 (D2R) containing medium spiny

neurons (MSN) in the striatum of rodents. The co-localization of GPR88 with DR1 and DR2 dopamine receptors suggest a role of GPR88 in schizophrenia.

1.7.3 Metabolic disorders

For many oGPCRs a role in metabolic disorders has been proposed. GPR21[140, 141], GPR26[142, 143], GPR27[144,145], GPR50[146,147] and GPR82[148,149] have shown to be involved in diverse ways in the reduction of body weight, food intake and increase in insulin sensitivity and glucose tolerance in mouse models. GPR26 has been proposed as a strong regulator of energy homeostasis through the control of hypothalamic AMP activated protein kinase (AMPK); GPR27 modulates pancreatic ß-cell function, its knockdown in these cells is responsible for the reduction of insulin promoter activity and glucose stimulated insulin secretion, a phenotype similar to the one of the GPRC5B knockout.

On the other hand, the hyper activation of GPRC5B impairs the insulin production in pancreatic ß-cells, resulting in a phenotype comparable to Type 2 diabetes. GPRC5B is therefore considered a possible target for diabetes therapy in order to restore a normal insulin secretory function in patients affected by Type 2 diabetes.

1.8 Ligand-independent functions of oGPCRs

The examples of the previous section on the physiological relevant roles of oGPCRs are mostly obtained by studying the effect of knockdown, knockout and overexpression of those receptors in murine models. For other GPCRs for which it is not possible to identify a physiological function by these genetic methods, hypothesis about their physiological roles are more difficult.

This is why it is has been theorized that some or more oGPCRs might have a ligand-independent function, or might be not functional at all[150,151].

1.8.1 Constitutive activity of oGPCRs

In some specific cases it is possible to identify signaling properties and physiological importance of oGPCRs by high levels of constitute activity.

Constitutive activity for GPCRs is common and is due to specific sequences in the receptor structure that stabilize the active form, permitting the interaction with. Examples of oGPCRs constitutive activity are GPR3[152,153], GPR6[134] and GPR12[154]. These oGPCRs all couple to Gα-s subunit, increasing the amount of cAMP in the intracellular matrix. These receptors are also an example of the large amount of information that the constitutive activity bring with it: all three oGPCRs are shown to mediate different neurological functions, like promoting neuronal survival by inhibiting apoptosis in various physiological conditions, enhance neurite outgrow and increasing cAMP levels during neurite elongations. These receptors have a function in memory and learning (GPR6
knockout mice shown locomotion and memory problems), as well as a role in regulating pain and analgesia induced by morphine and cocaine. GPR3 has been identified as a molecular target for neuropathic pain therapy and as part of the pro-opioid receptor system.

The degree of constitutive activity of GPCRs and oGPCRs depends also on the tissue specific levels of G-Proteins and ß-arrestin2, and on the level of proteins such as G-Protein coupled receptor kinase 2 involved in GPCRs internalization and recycling.

Taking in account the importance of the constitutive activity for deciphering signaling coupling and physiological relevance of oGPCRs, a more precise description of the mechanism has been proposed in order to predict constitutive activity also for other oGPCRs.

The study of GPR61[155] has demonstrated that removing the N-terminus from this oGPCR reduce in a sensitive way the magnitude of the signal supposed to be related to constitutive activity. This lead to the hypothesis of the existence of an N-terminus tethered ligand that might regulate receptor activation.

The high constitute activity for the previously mentioned oGPCRs can be triggered by structural properties of the receptor, stabilized in its active form, or by the presence in the native tissue of an endogenous ligand no yet detected with any of the currently available approaches. This was the case for two oGPCRs, the GPR40 and the GPR174[156,157,158]. The GPR40[159] is now known

as FFAR1, or free- fatty acid receptor 1, because it was discovered that its constitutive activity was actually triggered by the permanent occupation of its ligand-binding pocket by the endogenous free fatty acid 1.

1.8.2 Orphan GPCRs acting as co-receptors

The struggle to identify oGPCRs ligands might also reflect that some oGPCRs might have a truly ligand-independent mechanism of activation of their signal transduction. One possible function without the need of agonists is if they act as co-receptors, forming heterodimeric (or higher order) complexes with other oGPCRs, GPCRs or other proteins. The properties of GPCRs to heterodimerize and form functional complex has been shown mostly for receptors belonging to the Class C.

One example of this kind of interaction is the heterodimer between GABAB1 and GABAB2[160,161]: GABAB1 is responsible for ligand binding, while GABAB2 is then activating the G-Protein mediated signal cascade. In this case, the GABAB2 receptor can be seen as the oGPCRs that use the ligand binding process and the related conformational change of his heterodimer partner to enhance signal transduction through G-protein.

A similar process has been recently observed for GPR179 in the retina[162], which can heterodimerize with the glutamate receptor mGluR6[163]. The functional heterodimer is involved in the signal transmission from photoreceptors in the retina to ON bipolar cells in case of dim light conditions.

In some cases, the presence of an oGPCRs can interfere positively or negatively with the function and activity of an independent ligand-activated GPCRs: this is the case of the dimer complex between GPR50 and the melatonin receptor MT[164,165], where the role of GPR50 after formation of the dimers is to negatively interfere with the melatonin-depended signal by interaction with its long C-terminal tail. In a similar case, the ß-alanine binding mas-related receptor MRGD signal gets potentiated and its internalization inhibited by interacting with its orphan homolog MRGE[166].

It has been shown also that oGPCRs can form heteromeric complexes also with proteins other than GPCRs. GPR37 can associate with the dopamine transporter DAT, regulating its activity negatively. In knockout GPR37 mice dopamine uptake and DAT surface expression in striatal membranes are both upregulated[167, 168].

For some GPCRs it has been demonstrated the formation of complexes also with ion channels, such as the ß2-adrenergic receptor and the calcium activated potassium channel[169] or the calcium channel Cav1.2[170] or the complex between dopamine receptor D5 and the GABAa ligand-activated channel.

In the majority of these non-oGPCRs-ion channel complexes a ligand is not required since they are formed constitutively, suggesting that such complex might be also formed between oGPCRs and ion channels.

1.9 Experimental approaches to study G P C R

The modularity and shared topology of GPCRs allowed the development of experimental techniques that became fundamental for studying activation and signaling mechanisms. These techniques share the general property of improved temporal and spatial control of GPCR activation and signaling. This allows to dissect GPCR structural properties and partition common functional motifs and their role in physiological relevant processes.

One pioneering approach was the design, through targeted mutations, of GPCRs that respond only to synthetic ligands, called RASSL[171,172,173,174]. This approach permits to activate selectively GPCRs by removing ligand dependence on the endogenous agonist(s) present in the tissue in which GPCRs are expressed. RASSL receptors were used to demonstrate that in t h e taste signal processing differently activated cell populations play a fundamental role rather than the activation of specific GPCRs[175,176].

This approach has been further used and developed also in neuroscience with the creation of a class of synthetic GPCRs called DREADDs[177,178] able to be activated only by an inactive cloazapine derivative. Collectively, these synthetic receptors have been used to study neurological process at the level of the CNS

and in the peripheral nervous system. The previously described approaches are based on a deep physiological and structural knowledge of the receptor studied. For many GPCRs a limited amount of information is available and this limitation reduces the use of the previously described methods. Novel methods allow to combine well-studied GPCRs with other GPCRs for which less information is available, in order to use the first as tool to study activation and signaling mechanisms of the latter.

1.9.1 Chimeric GPCRs as their use as functional probes

A chimeric GPCR is a synthetic protein created with DNA engineering from two different GPCRs that combines characteristics belonging to both parent receptors.

The chimeric approach is based on the hypothesis that GPCRs consist in first approximation of two substructures[179]:

- The first is formed by the N-terminus, transmembrane domains and the extracellular loops (responsible for ligand binding).
- The second is formed by the intracellular loops (responsible for coupling with the G-Protein and activation of different intracellular signaling pathways).

Also, in this hypothesis these two substructures are topologically connected but functionally independent.

A direct consequence of this hypothesis is the possibility to create by DNA engineering a synthetic GPCR fusing ligand binding and G-Protein binding domains from two different GPCRs (i.e. GPR**A** and GPR**B**). This process would lead to the creation of a receptor that is able of being activated by GPR**A**-agonists and transduce GPR**B**-signal transduction. This approach has been used widely, with large library of synthetic receptor created and tested. Up to 2004 more than 100 chimeric GPCRs were cloned and verified to be expressed and/or functional[179].

Chimeric GPCRs have been engineered to determine which regions in the protein structure are responsible for the ligand binding process. Substitution of the N-terminus or of the extracellular loops and transmembrane domain has been used to modify the affinity of GPCRs to different agonists.

Substitution of intracellular loops and intracellular portion of transmembrane domains has been demonstrated to be effective in switching and tuning the coupling of chimeric GPCRs between the different G-Protein mediated signaling pathways. Initially limited to GPCRs belonging to the same family or class, the creation of chimeric GPCRs with parents GPCRs coming from different classes was first achieve by creating a ß2-adrenergic receptor-Frizzled2 chimera used to study the mechanism of WNT signaling, especially important in early development[180,181]. There are at the moment no theoretical, experimental or bioinformatics tools precise enough to predict if a chimeric GPCRs is going to have folding and/or expression or signaling issues. From literature, it looks important to preserve the two different substructures while assembling the chimeric receptor. There are examples in literature[182] in which mixing of transmembrane domains belonging to the parents GPCRs leads to not functional chimeric GPCRs. However, it has to be taken in account that more not functional chimeras might have been engineered and tested, but not published. The concept that the preservation of the two substructures enhances
the probability of conservation of the receptor functionality is in accordance with the available structural data that show how transmembrane domains are highly involved in the conformational changes related to GPCR and signal activation.

Another strength of the chimeric approach is the conservation of selectivity in the G-Protein binding process. It has not been shown till now an example in literature in which the creation of a chimeric receptor leads to the activation of a G-Protein mediated signaling pathway not belonging to the parent GPCRs that donate the intracellular sequences supposed to interact with the G-protein. In comparison with other approaches like targeted mutagenesis, the chimeric approach has the advantage to give a quantitative analysis in the change in

properties of the GPCRs such as possibility to bind to new ligands or to signal with different G-Protein mediated pathway.

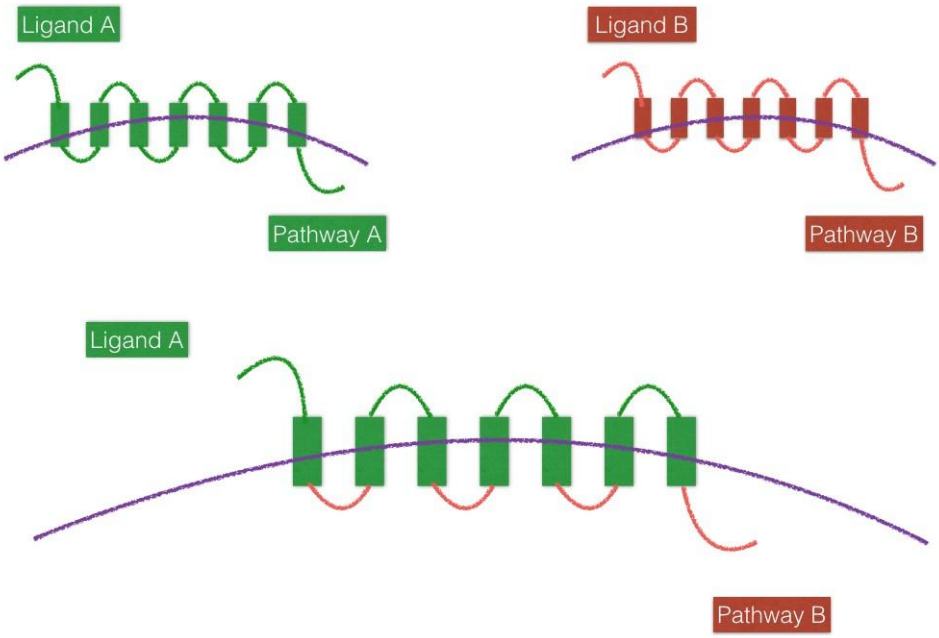

Figure 1.9 Chimeric approach for creating synthetic GPCRs with combined properties of parents GPCRs

1.9.2 Optogenetic control of GPCRs

Experimental approaches that lead to the control of GPCRs and their related G-Protein mediated signaling pathway beyond the use of endogenous or exogenous agonist or antagonist are fundamental for the study of the physiological processes that are mediated by GPCRs activation. Chimeric GPCRs can be powerful and useful to study those processes, but they might be subject to constrains when based on the use of a chemicals as trigger for the activation of the synthetic chimeric GPCRs. In particular, (i) chemicals are subjected to diffusion processes that influence experimental spatial and temporal resolution, (ii) may have collateral side difficult to predict and (iii) oblige to control for possible toxic effects.

Light is one possible stimulus that is not influenced by the previously described limitations (together with temperature[183], magnetic fields[184-187], ultrasonic

radiation[188-190]) and it has been largely use as trigger for controlling biological processes.

The advantages of using light to modulate biological processes are (i) light allows to precisely control in time the experimental conditions, making possible to divide an experiment in two different states (ON and OFF state) and facilitating the interpretation of the data collected. (ii) modern optical techniques make possible to highly control the delivery of light, with spatial resolution that permit single cells activation (iii) light can be tuned to reduce at the minimum the side effects and damages to the biological system studied (phototoxicity).

Optogenetics is defined as a set of techniques based on the use of light to control biological processes. Light has been used more and more widely to modulate biochemical and second messenger pathways. Not all system can be naturally modulated by light, and that in order to make an experiment controllable with light both genetic engineering and special experimental set up might be needed.

Three different approaches can be used in order to achieve optical control of a biological system:

- Insert a light-activated protein (i.e. Channelrhodopsin) and use its endogenous coupling properties to modulate the system activity.
- By using DNA engineering, modify in a light-sensitive manner one or more proteins involved in the process of study.
- Create light-sensitive chimeric GPCRs with equivalent signaling functions to the receptor on which control need to be achieved.

In the case of GPCRs all approaches have been used. Improved DNA cloning techniques are facilitating the use of the chimeric approach with several protein families and larger number of receptors[191].

Light-sensitive GPCRs naturally expressed in vertebrates and recently discovered microbial light-sensitive GPCRs represent a large ensemble of light-

sensitive proteins that can be use as template for the creation of light-sensitive chimeric GPCRs[192,193].

1.9.2.1 Light-sensitive GPCRs

The process in which the absorption of a photon from a protein activates a signal cascade is called phototransduction. Light-sensitive GPCRs are defined as opsins, and they represent the universal photoreceptor molecules of all visual system in the animal kingdom. The phototransduction process in the opsin is G-Protein mediated. Upon interaction with a photon (or a few photon) opsins undergo morphological changes that trigger the activation of G-Protein mediated signaling pathways[194].

The mammalian visual system is a good example to explain the mechanisms of phototransduction. Opsins are expressed both in cone and in rods (two different sets of photosensitive cells). Upon the opsin interaction with photons, the Gα subunit detach from the Gß-γ subunit, and binds to its effector cyclic nucleotide phosphodiesterase[195] (PDE). The activation by the Gα subunit of the PDE causes a decrease in intracellular concentration of cGMP, inducing the closing of cyclic nucleotide gated channels. Channel closure modifies the cell membrane potential, bringing the photosensitive cells to a state of hyperpolarization. Hyperpolarized cells released neurotransmitters to downstream cells, making the visual signal travelling till it reaches the ganglion cells that form the optical nerve, and as last step the brain.

Opsins can be divided in 6 subfamilies[196,197,198]:

- Vertebrate opsins
- Gα-o coupled opsins
- Gα-s coupled opsins
- Gα-q coupled opsins (found in invertebrates)
- Photoimerase opsins

- Neuropsins

The vertebrate opsins subfamily comprises visual and non-visual opsin with different spectral sensitivity, including four groups of cone opsin and one group of rod opsin. The four groups of cone opsins are divided according to the wavelength for which they are sensitive:

- The S group is sensitive to UV and violet light.
- The M1 group is sensitive to blue light.
- The M2 group is sensitive to green light.
- The L group absorbs red or green light and it can be consider strictly related to the opsins in the M2 group.

In the human genome, nine different genes code for opsins: one gene for a long wave sensitive opsin (OPN1LW), one gene for medium wave sensitive opsin (OPN1MW, with two isoforms OPN1MW2 and OPN1MW3), one gene for a short wave sensitive opsin (OPN1SW) and one coding for rhodopsin[199]. The remaining five genes code for opsin not involved in the human vision: OPN3 (also known as encephalopsin), OPN4 (melanopsin), OPN5 (neuropsin), RRH (peropsin) and RGR (a retinal coupled GPCR).

Several hypotheses have been made about the physiological role played by not visual opsins.

Encephalopsin (OPN3) was the first not-visual opsin discovered and it has the interesting feature of being expressed in cells and tissues classically believed not to be photosensitive. It is present in the brain (mostly in the hypothalamus) in the heart, lung, skeletal muscles, pancreas kidney and placenta. It is supposed to play a role in circadian rhythms and in not photosensitive processes such as asthma, and presents a peak of absorption in the blue-green region[200,201].

Melanopsin (OPN4) it is considered to be a Gα-q coupled opsin according to sequence analysis and experiments[202-204]. It presents an absorption maximum

around 480 nm and is mostly expressed in the photosensitive retinal ganglion cells (pRGCs). It also shows the interesting feature of having a bi-stable state, making the process of photoinduction reversible by light. pRGCs are involved in the stimulation of different brain regions such as the suprachiasmatic nuclei (SCN), the olivary pretectal nucleus (OPN) and the ventrolateral preoptic areas (VLPO).

It is supposed to be involved in the visual system, but not in the image-forming set of responses to light, but in processes such as the suppression of melatonin release, pupil constriction, circadian rhythms and related induction of sleep[205,206,207].

Neuropsin (OPN5) in expressed in the eye, brain, testes and spinal cord in human. It is listed as a Class A oGPCR, although it is known to respond to light in the UV range. In a three-state activation system, the ground state can be excited with UV wavelengths (absorption peak at 380nm) to bring the protein to an excited state, which is stable in the dark and can be further activated by blue light[208]. Another light excitation (with wavelengths in the range of 635-690 nm) can bring back neuropsin to its UV sensitive ground state. It has been demonstrated that UV activation of neuropsin can trigger coupling of the receptor with the Gα-I protein in mammalian cells, lowering the level of intracellular cAMP[209-212].

RRH, also known as peropsin, it is localized in the apical microvilli of the Retinal Pigment Epithelium (RPE), in proximity with the photoreceptor of the outer segment of the human visual system. RRH has the property to use as chromophore all-trans-retinal, and to isomerize it to the 11-cis form. This is the opposite process that usually occur in the phototransduction process (i.e. in rhodopsin), where 11-cis retinal is isomerized by light to its all trans form. This photochemical property suggest that RRH might be involved in the machinery of retinal recycling, isomerizing all-trans-retinal to 11-cis retinal that can bind to other opsin express in the visual system[213-215].

RGR, or Retinal G-Protein coupled receptor, is expressed mostly at the RPE, like RRH, but also in the Mueller cells of the neural retina. RGR has been

isolated and purified in the bovine RPE, showing both the properties to respond to UV and visible light and to bind to all-trans retinal but not to the 11-cis form. This, like in the case of the RRH, can suggest a role in the retinal recycling machinery, taking in account that the all-trans retinal get enzymatically bring back to their 11-cis form and supplied to other opsins[216,217,218].

1.9.2.2 Use of light-sensitive GPCRs in Optogenetic

There are several examples in literature of the use of endogenous or chimeric light-sensitive GPCRs.

The Korana group opened to the use of chimeric light-activated GPCR to control biological process, although their work does not contain any in vivo application[219]. In order to prove a common activation mechanism for GPCRs (based on the structural information available at the time) the Korana group engineered several rhodopsin-ß2-adrenergic receptor chimeras. For each of the

intracellular loops of the ß2-adrenergic receptor different candidate sequences to be cloned at specific sites of the rhodopsin structure were identified. In order to decide where to insert the intracellular loops of the ß2-adrenergic receptor in the rhodopsin sequence, the Korana group identified the boundaries between the transmembrane domain of rhodopsin and the intracellular domains of the ß2-adrenergic receptor. Cloning single and different combination of the ß2-adrenergic receptor intracellular loops in the rhodopsin sequence different versions of the rhodopsin-ß2 chimera were created. Comparing the light-stimulated signal with the one of the isopropanol activated ß2-adrenergic receptors it was suggested that the intracellular loops might have different roles in the coupling the ß2-adrenergic receptor with the G-Protein. The Korana group identified as minimal working chimera the one in which only the third intracellular loop of the ß2-adrenergic receptor is present in the rhodopsin structure, with only one sequence suited to create a functional chimera out of the six possible sequences for the third intracellular loop. The full rhodopsin-ß2- chimeric receptor, with all four extracellular loops belonging to the ß2- adrenergic receptor, showed activation half in magnitude in comparison with the

one obtained by stimulating the endogenous ß2-adrenergic receptor with 10 uM Isoproterenol.

In addition to this, the Korana group showed that the replacement of the third intracellular loop in rhodopsin was already sufficient to drastically reduce the rhodopsin-G-protein mediated signaling pathways (Gα-t1/2), while the replacement of other loops kept the signaling properties of rhodopsin intact. It has to be noticed that the results of the Korana group works have been later confirmed by the crystal structure of the ß2-adrenergic receptor solved by Rasmussen in 2011[21].

The work of the Korana group on the rhodopsin-ß2-adrenergic receptor chimera is extremely important for several reasons. First, they used for the first time the chimeric approach to prove a common activation mechanism between GPCRs and to verify their modularity based on the information available on rhodopsin. Second, they opened the way to the application of chimeric GPCRs in vivo by proving that they can trigger a response strong enough to be candidate for an in vivo use.

Third, they showed how delicate is the choice of the sequences for loops replacement and the likelihood of getting a not-functional chimeric receptor.

In 2009, the results achieved by the Korana group were used by the Deisseroth group to engineer a set of light-activated chimeric GPCRs[220], termed optoXRs. By sequence homology, Deisseroth and co-workers identified the same cutting sites in the sequence of the ß2-adrenergic receptor and the α1-adrenergic receptor, getting a single candidate sequence for both the intracellular loops of rhodopsin and the other two amine receptors.

In comparison with the work of Korana, the optoXRs of Deisseroth achieved a light stimulation of the same order of magnitude of their relative ligand-activated receptors, although a different (less potent) agonist was used (10uM of Norepinephrine instead of 10uM Isoproterenol). The further step proved by Deisseroth paper was that the signal obtained by light stimulation of optoXRs was strong enough to trigger reward-related behavior (in the case of the light-

activated α1-adrenergic chimeric receptor) and anxiety related behavior (in the case of the light-activated ß2-adrenergic chimeric receptor) in a mouse.

One other example of control of neuronal activity through the use of chimeric GPCR is represented by the rhodopsin-5-HT1a chimera engineered by Herlitze[221] group in 2010. The 5-HT1a is a metabotropic G-Protein coupled receptor linked to the Gα-i/o signaling pathway.

In this case only the insertion of the C-terminus of the 5-HT1a in the rhodopsin structure it was sufficient to light activate the G-Protein related inward rectifying potassium channel and to cause a membrane hyperpolarization in the hippocampal neurons, comparable to the agonist induced response of the endogenous 5-HT1a. Also, the light-activated chimera is able to rescue 5-HT1a signals in neuronal slices of knockout mice, proving the possibility to use such tool in controlling serotonergic neuronal activity and behaviors in normal and disease-related murine models.

The repertoire of opsin in vertebrate and human offers the possibility to explore different wavelength sensitivities, a property that get even more important in vivo where tissue light absorption plays an important role.

Rhodopsin is not the only light-sensitive GPCRs that has been used as optogenetic tools.

Among the non-visual opsins, OPN3 (encephalopsin) has been shown to regulate cell migration in human orbital fat stem cells trough activation of MAPK and to down regulate the anti-apoptotic pathway in 5-Fluorouracil sensitive hepatocellar Carcinoma, as well as play a role as regulator of cAMP signaling[222].

Melanopsin (OPN4) has been used to create a synthetic light- regulated transcription device in order to enhance blood-glucose homeostasis in mice, by regulating calcium release in the ER and PKC-related activation of TRPC calcium pump[223]. Neuropsin (OPN5) has been used in Neuro2a and HEK293 cells to regulate cAMP decrease, calcium release and MAPK[210] activation upon illumination with UV light. In the design of optogenetic tools based on light-sensitive GPCRs, the potency of the light-induced signal has to be taken in account in order to think of possible in vivo uses. Opsins that show a bleach

resistant property can be used to repeat and sustain in time a low magnitude signal in order to achieve a light stimulated signal strong enough to be able to trigger the same biological responses of the relative agonist activated receptor. This is the case of the box jellyfish opsin[224] (JellyOP). This opsin shows the property of trigger a reversible, high magnitude and reproducible increase of cAMP in mammalian cells, with both a spike-like behave in the case of flashed illumination and a repeatable sustained level in case of repeated stimulation. This suggests that the JellyOP can be used as a promising optogenetic tool to regulate Gα-s activity in mammalian cells and as a template for design of chimeric optogenetic tool.

1.9.3 Bioinformatics tool in GPCR functional characterization

The properties of GPCRs described in the previous sections (modularity, structural homology, ligand and G-Protein coupling mechanisms) have been mostly studied with either in vitro or in vivo techniques.

Modern technology actually allows us to use a third approach, an in silico approach, to explore the properties of GPCRs and ideally to predict specific characteristics of them such as candidate ligands or G-Protein coupling just by knowing the amino-acid sequence[225].

There is nowadays a large variety of bioinformatics tools specialized in different aspect connected with GPCRs: GPCRs predictors, ligand predictors, structural predictors and G-Protein coupling predictors.

The sequencing of a larger number of vertebrate genomes is increasing the number of homologues of known human GPCRs found in these vertebrates. The identification of new homologues, as well as the identification of new possible candidate genes for GPCRs is usually done based on sequences homology.

Multiple sequence alignments have been the base of GPCRs discovery and classification. Historically, Muscle and ClustalW2 have been the most used software to perform this kind of alignment, while now Muscle has been proven to be faster and more stable of other software alternatives[226-228].

In the last years the focus on using in silico tools for studying GPCRs has moved to the development of algorithm able to predict the coupling of GPCRs with one

or more G-Proteins. The in silico approach would give an estimate of the coupling based on sequence properties and supply a guide for experimental design to actually prove the coupling itself.

The idea behind the development of such coupling-algorithms is based on the modularity of GPCRs. The hypothesis is that specific amino acid sequences and topological features both in the receptor and in the G-protein structurally determine the GPCRs-G-Protein coupling. These features facilitate the interaction with a particular Gα (in most of the cases) subunit. Crystal structures data and studies based on chimeric receptors suggest that the sequences responsible for G-Protein coupling are located in the cytoplasmic side of the receptor. In particular, the intracellular loops are supposed to play a major role in the G-Protein coupling process. The difficulty in understanding how this coupling might be influenced by amino acid sequences relay on different factors mostly concerning the description of the forces involved in the GPCR-G-protein coupling (i.e. chemical, electrical, quantum) and at which scale these forces acts (intracellular level, single loop, single amino acid etc.).

All these factors imply that studying the GPCR-G-Protein coupling cannot be limited to the description of the interaction as a two bodies problem, but requires a statistical approach. GPCRs are classified in different families according to structural properties; the same structural properties may allow a classification of GPCRs according to their coupling properties that goes beyond the experimental evidences. Algorithms that can be trained on a sample of GPCRs for which the G-Protein coupling information is known represent the best available approach to guess coupling properties for GPCRs for which this information is still missing. The training sample is needed in order to create statistical fingerprints of known GPCR-G-Protein interaction based on structural properties. These fingerprints can be applied on GPCRs not included in the training sample and use to guess G-Protein coupling properties for these receptors.

One other approach would be to consider the probability of a specific G-Protein coupling given some peculiar structural properties (i.e. specific amino acids in the intracellular loops) on a subset of GPCRs for which the coupling is known, and extend these probabilities to the whole ensemble of GPCRs (including the

ones with unknown coupling).

These two different approaches are based on two different mathematical tools: hidden Markov Models to create GPCR fingerprints and Bayesian statistics to calculate conditional coupling properties. In its simplest description, a hidden Markov model is a stochastic model that assumes that future states of a system depend only on the present state that is partially observable. Bayesian statistics estimate the degree of belief in a proposition before and after accounting for evidence and compute a possible distribution of outcomes.

2-Thesis aims and hypothesis

In section 1.9 I described how orphan GPCRs have physiological relevant functions in many different biological process. A large number of oGPCRs are involved in different diseases and this make them promising future targets for drug development. The lack of endogenous or exogenous ligands makes the development of drug targeting oGPCRs a complicate process. The standard deorphanization approaches uses in the past years have achieved some success in the deorphanization process, but the rate with which new ligands are discovered for oGPCR has significantly slowed down.
The approach I proposed is based on the contemporary use of chimeric GPCRs and optogenetic. My aim is to find answer to three fundamental questions related to oGPCRs:

- Are oGPCRs functional at all?
- If yes, which signaling pathways do they preferentially activate?
- It is possible to retrieve a possible ligand for the receptors still consider as orphan?

For some of the oGPCRs these questions have been partially addresses and in some cases positively answered, but there is still a large number of oGPCRs for which this questions remain nowadays still without an answer.
Till April 2011 IUPHAR listed 94 oGPCRs in the Class A subfamily: out of these 94 receptors, 29 genes were either identified as opsins or insert in one GPCR group and named accordingly. For the remaining 65 receptors, the known information is gene sequences and expression pattern, while few data are available about other properties (putative ligands, signaling pathways and so on).
The previously mentioned three questions lead to a forth one: is there an alternative method to decipher activation properties and signaling pathway of oGPCRs and use this information to guess candidate endogenous agonist or to

design synthetic agonist?

My approach is based on the engineering of a complete light-activated library of chimeric receptor based on bovine rhodopsin with intracellular loops belonging to the 65 human Class A oGPCRs identified by the GPRXX nomenclature. This synthetic library shares the activation properties of bovine rhodopsin and would make possible to screen upon light activation the coupling properties of the selected 65 human Class A oGPCRs. A second library of light-activated chimeric receptors based on bovine rhodopsin and well-studied GPCRs will give the reference controls for light activation and G-Protein coupling of the light-activated oGPCRs.

Historically, the study of the coupling properties of GPCRs was mostly focus on the measurement of changes in concentration of second messenger such as cAMP, IP3, intracellular Ca^{2+} upon activation of different G-Proteins.

An ideal GPCR screening approach should be simple, nonradioactive, with a high signal-to-noise ratio, easy to handle (i.e. low number of reagents to add) and open for automation (i.e. use of 96/384/1536 well plate-formats). Screening for stimulation of cAMP by GPCR activation in generally straightforward[229-231] while use cAMP screening assay to monitor inhibition of cAMP generally required a more complicated approach (i.e. the use of foskolin for maximize the inhibition signal requiring preliminary titration experiments). Radiometric cAMP assays have been widely used, but are being replaced by fluorescence or luminescence-based assays. Alternative cAMP assays are based on ELISA (enzyme-linked immunoabsorbent assay) and FRET approaches. Intracellular Ca^{2+} can be monitored using calcium-sensitive fluorescence dyes (such as Fura2AM) and automated real time CCD based fluorescence plate readers[232,233]. Screening for G-protein mediated pathways that signal trough different second messengers can be as well screen using promiscuous G- proteins[36].

In case of studies based on a few number of GPCRs this approach might be still competitive, although its lack in universality.

In fact, the measurement of each different second messenger implies the development of different reading systems and experimental setups that makes difficult if not impossible to compare signal potencies for different G-Proteins even for single GPCRs. Screening for changes in the intracellular concentration of second messenger is an approach more sensitive to G-Protein mediated pathway cross talking, and introduces a not removable source of noise in the data.

The necessity of different experimental setups and analysis for each second messenger makes this approach extremely difficult in case of screening of large libraries of receptor. My approach is based on the screening of GPCRs coupling properties using a reporter gene system. Reporter gene systems are indeed more suited for multiple screening of receptors for different reasons. This approach is based on the production of a reporter gene response to activation of a specific signaling cascade (and relative second messenger). The magnitude of the activation can be estimated monitoring the expression levels of the selected reporter protein.

The activation of different subunits of the G-Proteins enhance the production of second messengers in the intracellular matrix and activates gene transcription by various response elements such as the cAMP response element (CRE), nuclear factor of activated T-cells response element (NFAT-RE), serum response element (SRE), serum response factor response element (SRF-RE, a mutant form of SRE) and several others.

Reporter genes systems have been used to screen GPCRs signaling properties linked to cAMP or Ca^{2+} signaling due to their stability and sensitivity. Despite the concern related to the distance between the activation event and the reporter protein expression, reporter gene systems have the clear advantage to share a common reading system for all gene activation, considering that the quantity measured in this set up is one unique reporter protein.

The optogenetic stimulation of the 65 light-activated human Class A oGPCRs and their screen using gene report systems will allow me to prove functional

properties and G-Protein coupling preferences for the selected human oGPCR genes and allow the functional dissection of their physiological role, increasing the information available in the finding and design of appropriate agonists for these genes.

3 - Material and Methods

3.1 Engineering of human Class A oGPCR light-activated chimeras

3.1.1 Algorithm for the design of chimeric GPCRs

I adopted as basic principle for the creation of the chimeric GPCRs the domain swapping principle. The domain swapping principle is based on the exchange of sequences corresponding to the intracellular loops of to the acceptor GPCR with the sequences corresponding to the intracellular loops belonging to the donor GPCRs genes. The approach I used to create rhodopsin-based light- activated chimeric GPCRs was systematically designed in order to be used “off the shelf”, i.e. not dependent on the number of genes taken in consideration.

I chose bovine rhodopsin as the light-activated GPCR template for the creation of light-activated human Class A oGPCRs and control GPCRs. I made this choice based on previously published work (see Section 1.9.2.2) in which bovine rhodopsin was successfully used to engineer light-activated chimeric GPCRs. This choice is further supported by the fact that the all the GPCRs for which I created light-activated chimeric versions belong to the same GPCRs Class A subfamily. Lastly, among all class A GPCRs rhodopsin presents the unique feature of having a negligible constitutive activity (or in this case dark activity) thanks to the ionic lock between its TM3 and TM6 (see section 1.4.2). One of the strengths of the chimeric approach is that there are no limitations on the choice of genes to be used as parent GPCRs. In my case the choice of rhodopsin is definitely the best option for all previous listed reasons. However, the same chimeric algorithm, as well as the cloning and the experimental procedures described in the following sections, could be used with a different GPCR as acceptor GPCR (i.e. ß2-adrenergic receptor). Of course a different acceptor GPCR would change the properties of the chimeric GPCRs, for example introducing higher levels of constitutive activity or functional selectivity.

I first compared the amino acid and nucleotide sequences of the donor GPCRs (oGPCRs and control GPCRs) with the bovine rhodopsin sequence. I assigned to each sequence a unique identifier and using Igor Pro the sequences were randomized in order and then aligned using MUSCLE algorithm[226,227].
The alignment of the donor GPCR sequences with bovine rhodopsin allowed me to identify regions belonging to intracellular loops of the donor receptors. I used the works of Korana and Deisseroth groups to select in the rhodopsin amino acid sequence:

- The cutting sites belonging to regions at the border of the rhodopsin transmembrane domains.

- The amino acid and nucleotide sequence of intracellular loops both for bovine rhodopsin and all donor GPCRs.

I identified for each donor GPCRs four candidate sequences corresponding to the four different intracellular loops (IL1, IL2, IL3, and IL4). I verified the candidate sequences for each intracellular loop of each donor gene by comparison with the information deposited in the Uniprot database.
The domain swapping can be achieved in at least two different ways:

- Design in silico of different light-activated chimeric GPCRs for ordering as synthetic genes.

- Design of a common rhodopsin-based plasmid and use high-throughput cloning techniques to cut and paste the intracellular loops belonging to the donor GPCRs in the acceptor plasmid (mother plasmid).

In this work I chose to use the second approach for several reasons:

- The creation of a mother plasmid allows the further creation of more light-activated chimeric receptors.

- Currently available gene synthesis techniques are still expensive in comparison with the order of a single mother construct plus short gene fragments.

One key requirement was that high-throughput cloning techniques can be improved and automatized in such a way to make the creation of large complete libraries of synthetic chimeric receptors achievable in reasonably short amount of time and limited resources.

3.1.2 High-throughput cloning

I designed a common mother construct for the cloning of light-activated chimeric GCPRs based on the domain swapping principle. This mother construct is mostly constituted by the nucleotides sequences belonging to the rhodopsin extracellular loops, transmembrane domains and the N - terminus. I inserted at the beginning of the mother construct sequences for membrane integration and an anti-VSV-G epitope for antibody staining (for the complete sequence of the mother construct see Appendix, Figure 6.3). The VSV-G epitope[234] represents the amino acid sequence YTDIEMNRLGK derived from the Vesicular Stomatitis viral glycoprotein. The epitope is located on the extracellular side of the mother construct and serves for checking membrane expression through antibody staining. In the rhodopsin sequence, in the same positions corresponding to the intracellular loops, I designed four artificial loops.

The artificial loops are designed in order be able to use the mother construct as template for the cloning of both the human Class A oGPCRs and the control GPCRs libraries. The cloning approach itself is based on the use of TypeIIs restriction enzymes and the Golden Gate-cloning technique[191].

TypeIIs restriction enzymes cleave directionally outside of their recognition sequence and they recognize sequences that are continuous non-palindromic. They are made of two distinct domains, one for DNA binding and the other for DNA cleavage[235]. They are thought to bind to DNA as monomers for the most part, but to cleave DNA cooperatively, through dimerization of the cleavage domains of adjacent enzyme molecules. For this reason, some TypeIIs

enzymes are much more active on DNA molecules that contain multiple recognition sites. The Golden Gate cloning technique is a high-throughput genetic engineering technique based on the use of Type IIs Restriction enzymes[236]. The presence of Type IIs restriction enzyme recognition sites in the DNA sequence that has to be cloned ("insert") in the acceptor plasmids implies multiple advantages in the cloning strategy:

- No restriction sites in the cloned gene (“seamless” cloning)
- Digestion and ligation happen in the same reaction step without the need for gel purification
- More than one insert can be cloned inside for each cloning step

The Golden Gate cloning approach had to be tailored to fit my needs. The sequence for the bovine rhodopsin-based mother construct was finalize considering the proposed cutting sites from Airan *et al*.[220]. Those cutting sites correspond to the start at the end of the transmembrane domains of bovine rhodopsin that enclose the intracellular loops (IL1, IL2, IL3 and IL4). The fake intracellular loops shared a common design with some specific difference among them in order to perform Golden Gate cloning:

- Each fake intracellular loop starts and ends with two sequences that are also present on each corresponding control and oGPCRs intracellular loop (i.e. every first intracellular loops of the control/orphan receptors will start and end with the same sequences present at the start and the end of the first fake intracellular loops in the mother construct).
- The start-end sequences are unique for each non-coding intracellular loop of the mother construct and this guarantee the conserved directionality in the insertion of the control/oGPCR intracellular loops.
- Each artificial loop contains a unique pair of TypeIIs restriction enzymes sites in opposing direction. The presence of these sites allow the

insertion of the control/orphan intracellular loops and does not make possible the re-ligation of the fake insert that would be re-digested.

- An additional and unique analytical enzyme site is present in each fake intracellular loop to eliminate the plasmid in which one of more artificial loops were get re-ligated.

Loop	Start Overhang	End Overhang	Restriction sites	Analytical sites
I	CGTC	CTCA	GAGACC, GGTCTC - BsaI	ACCGGT - AgeI
II	GGTG	GCCA	GAGACG, CGTCTC - BsmBI	GCTAAGC - BlpI
III	CGGA	CGCA	GTCTTC, GAAGAC - BbsI	GATATC - EcoRV
IV	CGC/CGT	ACA	GAAGAGC, GCTCTTC - SapI	GCGGCCGC - NotI

Tab 3.1 Restriction enzymes and relative sequences (different of each of the four fake intracellular loops) contained in the rhodopsin-based mother construct

I performed the screening of the light-activate oGPCRs and control GPCRs libraries in HEK293 (**H**uman **E**mbryonic **K**idney) mammalian cell lines. Considering the use of a mammalian based screening system, I subcloned when necessary control GPCRs (in their full-length and light-activated form) as well as the 65 light-activated human Class A oGPCRs in a modified pcDNA3.1(-) mammalian expression vector. There are currently no off-the-shelf available mammalian expression vectors that are free from endogenous restriction sites for TypeIIs enzymes in their sequence. The presence of these endogenous cutting site would make the use of TypeIIs restriction enzymes impossible leading to unplanned cutting of the plasmid during the cloning process.

For this reason, I modified pcDNA3.1(-) (from Invitrogen V79020) in its nucleotides sequence by site-direct mutagenesis to eliminate any endogenous TypeIIs restriction sites. In the original pcDNA3.1(-) sequences six endogenous TypeIIs restriction sites are present:

- BsaI (two sites)

- BbsI (one site)

- SapI (three sites)

I eliminated these six endogenous sites by single point mutation as follow:

Restriction Enzyme	Original Sequence	New Sequence
BsaI	gagacc	Gagaca
BbsI	gaagac	Gaagag
SapI	gaagagc	Gaagaac
SapI	gctc tc	gctcc G

Table 3.2 List of single point mutations used to modify endogenous TypeIIs restriction sites present in the pcDNA3.1(-) sequence

I subcloned the bovine rhodopsin-based mother construct in the modified pcDNA3.1 (-) using XhoI and EcoRI restriction sites. The intracellular loops belonging to the human Class A oGPCRs and control GPCRs were ordered in the form of oligonucleotides (Integrated DNA Technologies) and synthetic genes (Epoch Life Science).

In the case of the intracellular loops ordered as oligonucleotides, each intracellular loop came in the form of a forward and reverse oligonucleotide. Prior to insertion in the mother construct, I resuspended both oligonucleotides (forward and reverse) for each intracellular loop to a final concentration of 5ug/ul. I then mixed 0.5ul of each complementary oligonucleotide in 99ul of annealing buffer (10 mM Tris, pH 7.5-8.0, 50 mM NaCl, 1 mM EDTA in distilled water) and I heated the solution to 95°C for 15 min. Prior to use, I allowed the solution to slowly cool to room temperature. I performed the insertion of the intracellular loops in the mother construct using 96 well plates in order to being able to perform each cloning step in parallel for all light-activated human Class A oGPCR and control GPCRs. I tested different conditions in order to develop the optimal modified Golden Gate cloning protocol:

- Variation in the number of intracellular loops inserted for each cloning step.

- Variation in the number of incubation cycles for each cloning step.
- Variation in the duration of each single incubation cycle.
- Role of the analytical digest in the intracellular loops insertion efficiency.
- Role of the restriction enzymes heat inactivation in the intracellular loops insertion efficiency.

In the final set of conditions, I prepared the following mixture with a total reaction volume of 20ul for each cloning step (two for a total of four loops exchanged for each chimeric GPCR):

1. 100 ng uncut plasmid DNA (i.e. mother construct)
2. 7.8 ng each intracellular loops
3. The ratio between the mother construct plasmid and the intracellular loops was approximately 5:1
4. 1ul T4 DNA ligase
5. 2ul of Promega ligation buffer
6. 5 units of the first TypeIIs restriction enzyme, depending on the intracellular loops chosen

I then incubated the mixture in a thermocycler for a first set of heating cycles:

Number of cycles	First time step	Temperature	Second time step	Temperature
11	2 minutes	37 degrees	2 minutes	37 degrees

Table 3.3 First set of thermocycles with duration and temperature of each cycles and total number of cycles

At the end of these first set of thermocycles, I added five units of the second restriction enzymes (always in accordance with the chosen intracellular loops), and started a second set of thermocycles:

Number of cycles	First time step	Temperature	Second time step	Temperature
11	2 minutes	37 degrees	1 minute	37 degrees

Table 3.4 Second set of thermocycles with duration and temperature of each cycles and total number of cycles

After the second set of thermocycles I heat inactivated the TypeIIs restriction enzymes by increasing the mixture temperature to 50°C for 5 min and up to 80°C degrees for 10 min. I let cool down the mixtures at room temperature for at least half an hour. In order to reduce the amount of clones containing mother construct still having the artificial intracellular loops, I digested the PCR products with 5 units of an additional restriction enzyme (AgeI, BlpI, EcoRV or NotI corresponding to IL1, IL2, IL3, IL4).

I retransformed the digested PCR products into E. coli XL10 gold competent cells. In order to keep the 96 well format and the high-throughput approach of the cloning technique, I added 50ul E. coli XL10 gold competent cultures to each well of a 96 well plate PCR plate. I tested an aliquot of the cells need for each plate for competency by retransforming 1ng of pcDNA3.1(-) in 1ul and counting the number of colonies. I only continued with cultures/plates that yielded more than 1000 colonies for transforming the Golden Gate PCR products after storage at -80°C. I transformed Golden Gate PCR products using the 96 well PCR E. Coli XL10 competent cells plates and plated them in six well dishes I previously filled with LB media and ampicillin. I used glass beads in order to speed up the plating process (~3-4 glass beads for each well of the six well plates). After transforming, I miniprepped 2 clones for each Golden Gate PCR products that were verified by Sanger sequencing (LGC Genomics). I selected only one positive clone for creating the libraries. The modified Golden Gate approach was generally extremely stable with an efficiency grated than 80%. The modified Golden Gate approach worked for most of the genes. In some specific cases, it was more difficult and in some other cases not possible to obtain positive clones using the general approach described before. In those cases, I applied modifications to the established protocol in order to increase intracellular loops insertion efficiency. One first modification I performed was to

add two intracellular loops in two different moments: first intracellular loops and relative TypeIIs restriction enzyme before the first set of thermocycles and the second intracellular loops with relative TypeIIs restriction enzymes before the second set of thermocycles. Also, since there are not constrain on the order of which intracellular loops can be inserted into the mother construct, I tested all possible permutation of two loops insertion. Both previous modifications allowed me to increase the number of positive clones obtained for the light- activated chimeric libraries. For some of the remaining genes that still presented difficulties in cloning, I engineered an alternative modified Golden Gate protocol, based on the principle of reducing the percentage of glycerol from the restriction enzymes in the total volume of the initial mixture. I set the total reaction volume to 32ul, consequently increasing the amount of Promega Ligase buffer to 3.2ul. I kept the quantity of mother construct, intracellular loops and relative ratio unmodified. This modified protocol allowed to test also the role of the analytical digest in the previously described cloning modified Golden Gate cloning protocol. I retransformed 1.5ul of the mixture prior digestion and I digested the remaining 30.5ul using the appropriate analytical enzymes as already described according to the standard Golden Gate protocol. The result was not conclusive, but no major improvement was reached when the analytical digest was omitted. For a few amount of light-activated human Class A oGPCRs both the original and the modified Golden Gate protocols did not give positive clones. In this case, in order to preserve the completeness of the light- activated orphan library, I ordered the corresponding genes were prepared using gene synthesis (Epoch Life Science).

3.2 Functional testing of light-activated chimeric GPCRs

3.2.1 Luciferase-based reporter plasmid for screening G-protein mediated signaling pathways

For the screening of GPCRs I used a gene reporter system based on different luciferases. This system has the advantage to be easily identifiable and quantifiable using the bioluminescence properties of the enzyme luciferase when interacting with the appropriate substrate luciferin. Among luciferases present in nature, the luciferase from Photinus pyralis (Firefly luciferase) and from Renilla reniformis (Renilla Luciferase) have been widely used in gene reporter systems considering the full characterization of their enzymatic reactions[237]. Luciferase-based gene reporter systems have the ability to test many signaling events by coupling a response element linked to the signal that has to be detected to the transcription of the luciferase gene. The intensity of the signal can be measured and quantified by bioluminescence. For GPCRs it is known that signal transduction mediated by the interaction with different Gα subunits regulate different intracellular second messengers. Increase or decrease of intracellular level of these second messengers can up-or down-regulate gene transcription.

More specifically, in case of GPCRs the relation between second messengers and gene activation is well known and can be in good approximation described as follow[238]:

- Activity of GPCRs that modulate increase or decrease of intracellular concentrations of cAMP can be monitored by coupling luciferase transcription to a cAMP responsive element (**CRE**) [239,240]
- Activity of GPCRs that modulate increase of intracellular concentration of calcium trough IP3 (Ca^{2+}) can be monitored by coupling luciferase transcription to the transcription factor Nuclear factor of activated T-cells (**NFAT**) [241,242]

- Activity of GPCRs that interact with RhoGEF and RhoA can be monitored by coupling luciferase transcription to a modified serum response factor, sensitive only to RhoA activation (**SRE.L**)[243]

- Activity of GPCRs that modulate activation of the ERK1/2 pathway can be monitored by coupling luciferase transcription to the serum responsive element (**SRE**)[244]

The previous description is limited to the interaction between second messengers and transcription factors. To extend this description to the interaction between G-protein mediated pathways and transcription factors, it has to be taken in account the complexity of the G-protein mediated signal transduction. This complexity is actually an advantage, because it permits to have different choices of transcription factors to couple to luciferase to screen the same G-protein mediated pathway.

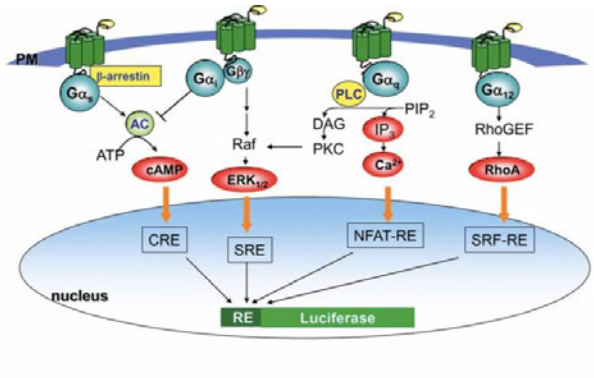

Figure 3.1 Mechanism of coupling between G-protein mediated pathways and luciferase gene response systems through different transcription factors (from Cheng *et al.*[238])

For example, in case of Gα-q coupled GPCRs, their activity can be monitored using a NFAT responsive element (that will respond to raise of intracellular Ca^{2+} concentration trough IP3), or using a SRE responsive element (that will respond to PKC and consequent ERK1/2 activation). I decided to use three different Luciferase based reporter plasmids:

1 - A CRE reporter plasmid (Pgl4.29, Promega) sensitive to change in cAMP concentration and related to activation of Gα-s mediated signaling pathways.

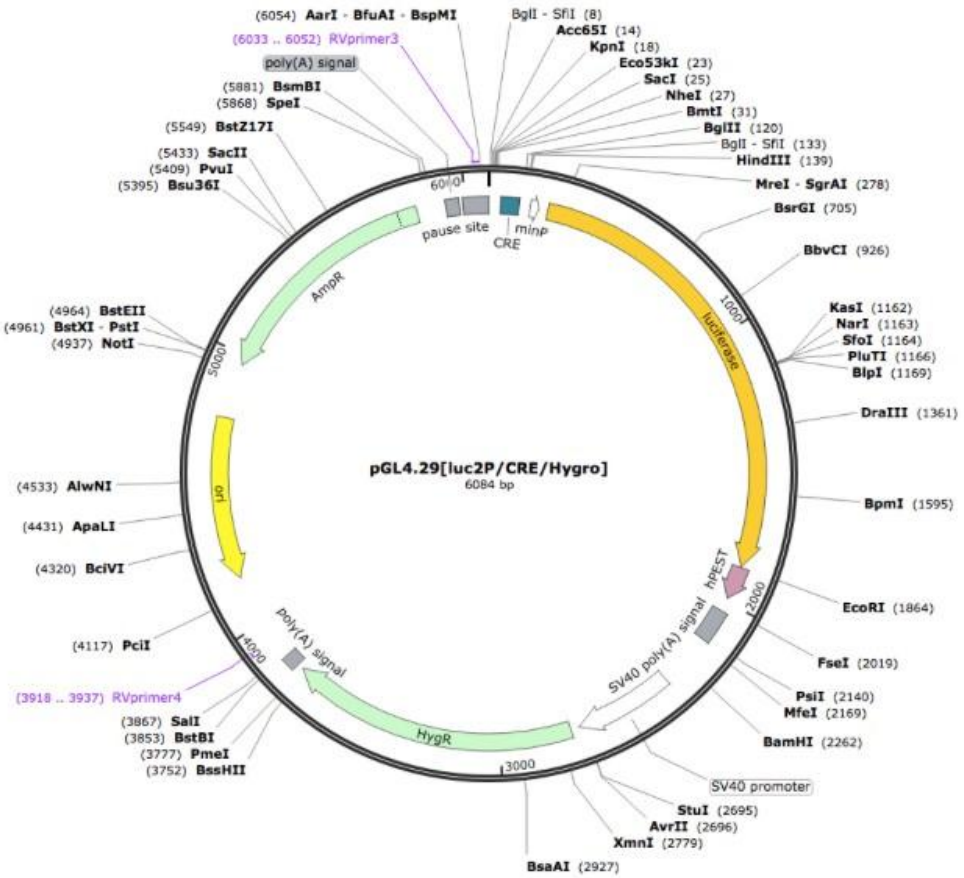

Figure 3.2 Vector map of the luciferase-based CRE reporter plasmid

2 - A SRE reporter plasmid (Pgl4.33, Promega) sensitive to activation of MAPK trough β-arrestin and PKC and hypothetically related to activation of Gα-q and Gα-12/13 mediated signaling pathways.

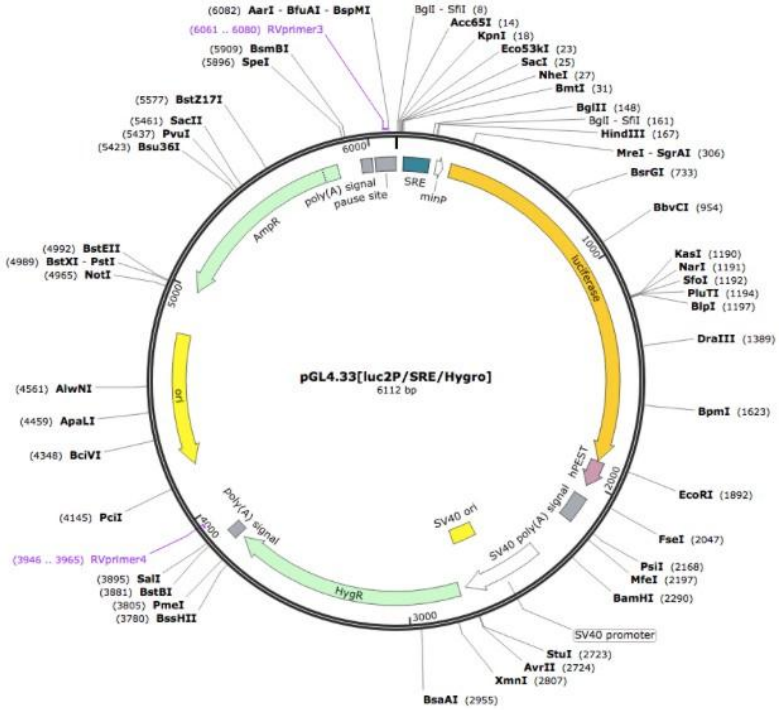

Figure 3.3 Vector map of the luciferase-based SRE reporter plasmid

3 - A SRE.L reporter sensitive to RhoA activation and hypothetically related to activation of Gα-q mediated signaling pathways

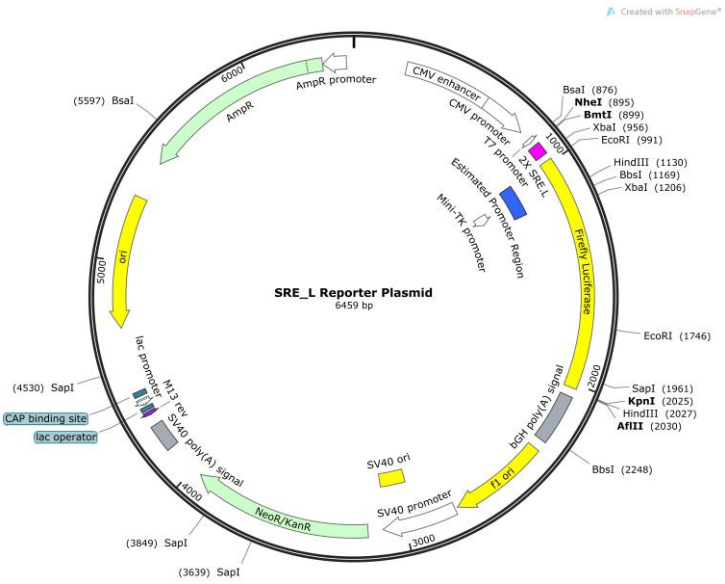

Figure 3.4 Vector map of the luciferase-based SRE.L reporter plasmid

I also used the CRE reporter to measure decrease in cAMP concentration by stimulating cAMP production using endogenously expressed receptors and estimate Gα-i/o mediated signaling pathways. All previously described reporter plasmids are based on Firefly Luciferase as reporter protein, which expression changes only upon activation of one of the previously described G-protein mediated signaling pathways. Gene reporter systems based only on a single reporter protein (such as Luciferase) might be statistically noisy by not taking in account hidden variables such as transfection efficiency (especially when not working with stable cell lines) and cell number. To get an estimate of these hidden variables, I co-transfected together with the Firefly-based Luciferase reporter plasmid a second Luciferase reporter plasmid based on Renilla Luciferase. I subcloned the Renilla Luciferase reporter gene in pcDNA3.1(-) (between NheI and XbaI sites) and thus it contains the same promoter of the Firefly based reporter plasmids, with the difference that is constantly expressed proportionally to the number of positively transfected cells and it is not affected by activation of any G-protein mediated signaling pathways.

3.2.2 Experimental procedure

I screened all genes included in this study with one single reporter plasmid at time. The total duration of each experiment was 5 days. On DAY 1, I treated 96 well plates with transparent bottom (Greiner Bio- one) with poly-l-ornithine (PLO) in dilution 1: 25 in PBS (final concentration 1mM). I added 70ul of the PLO dilution to each 96 well plate, and either incubated at 37° for three hours or stored in the fridge overnight. Plates coated with this procedure could be used for as long as four weeks keeping their coating properties. Before cell seeding, I washed the 96 well plates with PBS in order to remove any residual PLO. When removing PLO (as well as for any other experimental steps concerning the change of media in the 96 well plates) I used a cell culture tip (volume 250ul) attached to a cell culture aspiration pump. I used the tip instead of a classic glass pipette for its larger sucking section that lower the sucking pressure at the contact between the tip and the

well plate and reduce both the amount of coating aspirated and the amount of cell detached during the different media changes. I seeded four 96 well plates with HEK 293 cell at the concentration of 50000 cells for 100ul (well). I cultured and seeded HEK293 cells in DMEM supplemented with 10% FBS, 100 U/ml penicillin and 0.1 mg/ml streptomycin in a humidified incubator (37 degrees Celsius, 5% CO_2).

24 hours after seeding (DAY 2) I changed the media to antibiotics-free DMEM supplemented with 5% FBS. The choice of antibiotic free media was due to the fact that cells were transfected by lipofection and antibiotics are proved to lower transfection efficiency and cell viability. Transfection was performed following a template 96 well plate that was applied to all four 96 well plate seeded with HEK293 cells. In the template each well corresponds to either:

- A single light-activated human Class A oGPCR
- A single light-activated chimeric control GPCR
- A single full-length agonist activated control GPCRs

Transfection was performed on a 96 well plate using lipofection and polyethylenimine solution (PEI, 1mg/ml in H_2O; Polysciences) as lipofection reagent. I tried different ratios between receptor plasmids, Firefly reporter plasmid and Renilla plasmid in order to achieve the best combination in terms of transfection and reporter efficiency. For PEI best transfection efficiency was reached for 100ng of DNA for 1ul of reagent.

I also tried different ratio between the receptor plasmids, the Firefly reporter plasmid and the Renilla plasmid, leading to the final choice of keeping a constant ratio of 1:1 between the receptor plasmid and the Firefly reporter plasmid and a 10:1 ratio between the Receptor-Firefly reporter plasmids and the Renilla plasmid.

Taking in account the previously described constrains, for each well, I mixed 75ng of receptor, 75ng of Firefly reporter plasmid and 7.5ng of Renilla plasmid with 25ul of Optimem-L and incubate for 5 minutes. In parallel I diluted 1ul of

PEI solution (1mg/ml in H_2O; Polysciences) in 25 ul Optimem-I (Life Technologies 519043) for each well. I combined the two solutions, incubated at room temperature for 20 min and then added to the cells using a multichannel pipette. After six hours, I changed the culture media to starve media (DMEM supplemented with 0.5% FBS and antibiotics).

On DAY 3, I incubated the cells overnight with 10uM of 9-cis retinal (Sigma) dissolved in DMEM supplemented with 0.5% FBS and antibiotics. The binding of rhodopsin with 11-cis retinal and its isomerization in all-trans form upon interaction with a photon is a necessary condition for the activation of the rhodopsin signal cascade. It has been shown in literature that HEK 293 cells contains the machinery for retinal recycling, but it is not yet clear if the level of endogenous 11-cis retinal contained in the HEK293 cells would be sufficient for activate light-stimulated chimeric receptors. 9-cis retinal is functionally equivalent to 11-cis retinal and allow rhodopsin activation upon light stimulation[245]. On DAY 4, I performed light and ligand stimulation. Out of the four plates prepared and transfected, I chose two plates as light plates and two as dark plates. Prior to start light and ligand stimulation, I changed media to CO_2 independent media supplemented with 0.5% FBS and antibiotics. I performed ligand-stimulation of control receptors and reporter-only transfected cells right before light stimulation with selected agonist and fixed concentrations. I carried out ligand and light stimulation in an incubator (Exoterra) modified with 450 light emitting diodes (300 LED IP65, SMD3528; 150 LED IP66, SMD5050, 465-470 nm and 525-530 nm wavelength ranges). The four 96 well plates were ligand and light stimulated at 37 degrees Celsius for six hours with blue and green light (400 $\mu W/cm^2$) or only blue light (280 $\mu W/cm^2$, for the SRE reporter, in order to avoid endogenous activation of green-sensitive opsin expressed in HEK293 cells). I shielded the two dark plates from light by wrapping them in aluminum foil. When testing for reduction of intracellular cAMP concentration using the CRE reporter plasmid, I stimulated the cells with NECA (500 nM) immediately (three to five minutes) before agonist

and light stimulation in order to increase intracellular levels of cAMP by activating endogenously expressed adenosine receptors (A2A, A2B)[246].

Receptor(s)	Ligand	Final concentration
α1AR β2AR	Norepinephrine (NOR)	10 μM[220]
FFR3	Propionate (PPA)	10 mM[247]
A2AR	Adenosine (NECA)	100 μM[248]
D1R D2R	Dopamine (DOP)	100 μM[249],[250]
M2R M3R	Muscarine (MUSC)	100 μM[251]
M1R	VU357017 (positive, allosteric modulator)	25 μM[252]

Table 3.5 List of agonist used for the ligand stimulation of endogenously expressed GPCRs and transiently transfected full-length control GPCRs

After six hours of (ligand and light) stimulation, I developed luminescence signals using the Dual-Glo system from Promega. The protocol I used for the Dual-Glo Luciferase assay was slightly modified in comparison with the one proposed from the manufacturer. Prior to use the Dual-Glo Promega kit, I removed the CO2 independent media using the previously described procedure; I prepared the first reagent by mixing Firefly Luciferase buffer with the relative substrate. This buffer allows cell lysis and furnishes a proper substrate for reading Firefly luciferase values. I added 50ul of the Firefly substrate plus buffer to each well and I shook the plates manually plates for 10 minutes (covered with an aluminum foil to protect from light) in order to allow a complete lysis of the cells. I read Firefly values in a microplate reader (Biotech Synergy H1) using a luminescence fiber; intensity was integrated for 1 s for each measurement and I used an artificial software gain of 135 for every measurement.

After the Firefly measurement, I prepared the second reagent by mixing the Promega Stop&GLO substrate and buffer. 50ul of the mixture were added directly in the original 50ul of Firefly mixture. The STOP&GLO buffer has the

property to quench the Firefly luciferase and to act as a buffer for the Renilla luciferase. I shook again the plates for 10 minutes and I read Renilla values using a microplate reader with the same setting already described for the Firefly measurements.

3.3 Data Analysis for the luciferase-based screening platform

For the analysis of the luciferase assays, I normalized all Firefly values by the Renilla values for each well of the 96 well plates to take in account variations coming from cell number and transfection efficiency[253]. For each of the 96 well plates, I normalized the Firefly/Renilla values of each well with the average value of the non-agonist-stimulated control wells to consider the intrinsic variation of each plate and being able to compare between different plates.

I repeated each experiment three to five times. For each experiment, every 96 well plate was validated by first looking at the ratio of the agonist-stimulated control GPCRs versus the not agonist-stimulated control GPCRs. These controls were included on each plate and used as validation: if agonist control receptor did not show the expect induction between agonist and not agonist stimulation, the single plate was not taken in account in the overall experimental analysis for the specific reporter gene.

I used induction values calculated for light-activated control receptor as validation for the light-stimulation procedure. Average values were calculated for light-activated control GPCRs on both light plates (N=2) and dark plates (N=2). If light-activated control GPCRs did not show the expect induction between light state and dark state, the experiment was consider as not validated and not included in the overall average for the specific reporter plasmid.

I calculated averages of Firefly/Renilla ratios for each well on the total number of experiments for each different G-protein coupled pathway reporter plasmid. I used these values to identify orphan genes for which light activation was considered significant by calculating induction values defined as the ratio between Firefly/Renilla in light condition over dark condition.

I considered oGPCRs genes for which induction values were bigger than 2.5-fold as active (for CRE, SRE and SRE.L reporter plasmids), while for the NECA-stimulated CRE reporter plasmid significant light dependent induction was considered for genes in which values were lower than 0.7-fold.

3.4 Antibody staining of the light-activated chimeric oGPCRs

For antibody staining, I used a 96 well plates format following a similar procedure as described already in the previous section for the Luciferase assays. On DAY 1, I coated and seeded cells into a 96 well according to the protocol for the Luciferase functional assays. One DAY 2, I transfected all 65 light-activated Human Class A oGPCRs constructs plus rhodopsin as positive staining control following the procedure already described in the previous section. I considered not transfected cells plus primary antibody as negative staining control.

On DAY 3, cells were washed with DPBS (LifeTechnologies Inc.) two times for three minutes to remove any residual media and to decrease the chances of detach them in the following washing steps. I prepared 4% PFA solution from 95% PFA powder (Sigma) in PBS, and I used it to fix cells for 15 minutes at room temperature. PFA was used as fixation agent in order to not permeabilize the cell membrane; the not permeabilization of the cell membrane together with the location of the VSV-G epitope on the extracellular side of the mother construct allowed me to stain chimeric receptor that are expressed at the cell membrane. After fixation, I washed the cells four times for five minutes with DPBS; cells were later incubated 30 minutes in blocking buffer (1% BSA in DPBS, 50 ul/well) prior the incubation with primary antibodies (clone P5D4, Sigma, 1 hours at RT, 1:250 final dilutions in blocking buffer). I washed again the cells 3 times for 5 minutes' prior incubation with 30ul/well of anti-polyvalent biotinylated antibody for 10 minutes at RT.

After another washing step (2 times for 3 minutes with DPBS) I incubated cells with horse-radish peroxidase secondary antibody for 10 minutes at RT. I prepared a DAB mixture by adding 30 ul of DAB (Sigma, D6065) in 1 ml of DAB substrate (Sigma, D6190). After washing with DPBS (2 times for 3 minutes), I

added 40ul/well of DAB mixture and let incubate for 3 minutes at RT. I stopped the reaction adding 70ul/well of distilled water and I measured absorbance values at 450nm using a Synergy H1 plate reader for each well.

3.5 Bioinformatics tools for GPCRs-G-protein coupling prediction

At the moment in which this thesis is written, these are the algorithm published that claim to be able to decipher G-protein coupling for GPCRs:

- Cao, 2002: A **Naïve Bayes** model[254]
- Sreekumar, 2004: **hidden Markov** model [255]
- Yabuki, 2005: **hidden Markov** model plus support vector machine [256]
- Sgourakis, 2005: **hidden Markov** models and artificial neural networks [257]
- Sgourakis, 2005: refined **hidden Markov** model[258]
- Ono, 2006: **hidden Markov** model[259]
- Guo,2006: Autocross-Covariance Transform [260]

Some of these algorithms are available online, for the ones not published on public servers the authors were contacted in order to retrieve the original source code. The algorithms retrieved and used for this current work are:

- Yabuki, 2005: **hidden Markov** model plus support vector machine
    - GRIFFIN
- Sgourakis, 2005: refined **hidden Markov** model
    - PREDCOUPLE
- Sgourakis, 2005: **hidden Markov** models and artificial neural networks
    - PREDCOUPLE2

These algorithms are based on Hidden Markov Models. In order to have an estimate of how good these algorithms perform on data set of well-known GPCRs there are two main parameters to take in account: the sensitivity of the algorithm and the specificity. Sensitivity is defined as the number of positive results confirmed as positive from experimental data, specificity is the number of negative results confirmed as negative by experimental data. In the case of GPCRs coupling, sensitivity is the ability to predict a G-protein coupled pathway for a GPCR that has been confirmed by experimental approaches, specificity take in account the number of predicted G-protein coupled pathways that are not verified in experimental data.
For the chosen algorithms:

- The **Griffin** algorithm is trained on a set of 682 GPCRs (of which 394 are olfactory receptors) and has a claimed sensitivity and specificity of on average more than 85 % on the training set

- The **Predcouple** algorithm is trained on 282 GPCRs and a “correct classification rate” of 91 %, predicting 6 out of 24 experimental verified promiscuous GPCRs coupling

- The **Predcouple2** algorithm is trained on 226 GPCRs with a claimed sensitivity of 100% and specificity of 92 % on the training dataset.

For all these algorithms, two different data set were prepared:

- One dataset containing well-studied GPCRs (overlapping with the training dataset of each algorithms) and the original sequences of the 65 chosen oGPCRs

- One dataset containing the sequences of the 65 light-activated Human Class A oGPCRs

I collected data for each of the dataset. For each control and oGPCR for which the algorithms were run I considered each pathway with a probability of coupling higher than 50 % as predicted coupling.

4 - Results

4.1 Human Class A oGPCR gene selection

94 genes in the human genome are currently listed and classified as human Class A oGPCRs. For all class 94 human Class A oGPCRs I retrieved both amino acid sequences and nucleotide sequences from the Uniprot database (http://www.uniprot.org). For GPR79 it was not possible to retrieve a deposited nucleotide sequence and it was excluded from the study. For a limited amount of genes, the nucleotide sequences were adapted to match the deposited protein sequence. I did not include 29 human oGPCR genes in this study either because they are currently considered as opsins (OPN3, OPN5) or because classified in one of the GPCRs subfamilies.

In this study I selected 65 genes for engineering of bovine rhodopsin-based chimeric receptors. These genes correspond to the human Class A oGPCRs genes currently identified by the GPRXX nomenclature according to the IUPHAR (I first checked the list in April 2011 and verified it again in April 2015 to keep track of any changes).

Using the algorithm described in the section 3.1, I designed and cloned for all selected 65 human Class A oGPCRs their respective light-activated chimeras based on bovine rhodopsin.

For one gene, GPR101, I was not able to retrieve a meaningful chimeric sequence and therefore this gene was excluded from the functional screening.

Identifier	Gene name	SwissProt/Uniprot ID
MO3	GPR1	P46091
MO4	GPR3	P46089
MO5	GPR4	P46093
MO6	GPR6	P46095
MO7	GPR12	P47775
MO8	GPR15	P49685
MO9	GPR17	Q13304
MO10	GPR18	Q14330
MO11	GPR19	Q15760

MO12	GPR20	Q99678
MO13	GPR21	Q99679
MO14	GPR22	Q99680
MO15	GPR25	O00155
MO16	GPR26	Q8NDV2
MO17	GPR27	Q9NS67
MO18	GPR31	O00270
MO19	GPR32	O75388
MO20	GPR33	Q49SQ1
MO21	GPR34	Q9UPC5
MO22	GPR35	Q9HC97
MO23	GPR37	O15354
MO24	GPR37L1	O60883
MO25	GPR39	O43194
MO26	GPR42	O15529
MO27	GPR45	Q9Y5Y3
MO28	GPR50	Q13585
MO29	GPR52	Q9Y2T5
MO30	GPR55	Q9Y2T6
MO31	GPR61	Q9BZJ8
MO32	GPR62	Q9BZJ7
MO33	GPR63	Q9BZJ6
MO34	GPR65	Q8IYL9
MO35	GPR68	Q15743
MO36	GPR75	O95800
MO37	GPR78	Q96P69
MO39	GPR82	Q96P67
MO40	GPR83	Q9NYM4
MO41	GPR84	Q9NQS5
MO42	GPR85	P60893
MO43	GPR87	Q9BY21
MO44	GPR88	Q9GZN0
MO45	GPR101	Q96P66
MO46	GPR119	Q8TDV5
MO47	GPR120	Q5NUL3
MO4	GPR132	Q9UNW8
MO49	GPR135	Q8IZ08
MO50	GPR139	Q6DWJ6
MO51	GPR141	Q7Z602
MO52	GPR142	Q7Z601
MO53	GPR146	Q96CH1
MO54	GPR148	Q8TDV2
MO55	GPR149	Q86SP6
MO56	GPR150	Q8NGU9
MO57	GPR151	Q8TDV0

MO58	GPR152	Q8TDT2
MO59	GPR153	Q6NV75
MO60	GPR160	Q9UJ42
MO61	GPR161	Q8N6U8
MO62	GPR162	Q16538
MO63	GPR171	O14626
MO64	GPR173	Q9NS66
MO65	GPR174	Q9BXC1
MO66	GPR176	Q14439
MO67	GPR182	O15218
MO68	GPR183	P32249

Table 4.1 List of oGPCRs selected for the luciferase-based functional screening with relative identifiers and SwissProt/Uniprot reference numbers

4.2 Control GPCR gene selection

There are currently several GPCRs for which both several agonists and G-Protein mediated activated signaling pathways are known. I chose a subset of control GPCRs taking in account coupling properties and available information in literature on their use in a chimeric form. I chose nine different well studied GPCRs as candidate genes for the creation of light-activated control chimeric GPCRs.

These genes were also used in their agonist-activated form to estimate the efficiency of their respective light-activated chimeras and to prove the preservation of coupling properties between agonist and light-activated control GPCRs.

The genes I selected as full-length control GPCRs (defining as full-length receptors being able to be chemically stimulated by their proper agonists) belong almost entirely to the human genome, with the only exception of the α1 adrenergic receptor (α1AR) whose original species is rat.

Receptor	Species	Predom. G coupling.	Expr. Vector	Source
β2-adrenergic receptor (β2AR)	Human	Gα-s	pcDNA3	Robert Lefkowitz (Duke Univ.) *via* Addgene, Inc. (14697)
α1-adrenergic receptor (α1AR)	Rat	Gα-q	pCMV5	Robert Lefkowitz (Duke Univ.) *via* Addgene, Inc. (45760)

Free fatty acid receptor 3 (FFR3)	Human	Gα-i/o	pcDNA3	Graeme Milligan (Univ. of Glasgow)
Adenosine A2A receptor (A2AR)	Human	Gα-s	endogenous	Atwood 2011
Dopamine receptor D1 (D1R)	Human	Gα-s	pcDNA3.1(-)	MGC (MHS6278-202856822)
Dopamine receptor D2 (D2R)	Human	Gα-i/o	pcDNA3.1(-)	MGC (MHS6278-202830262)
Muscarinic acetylcholine receptor M1 (M1R)	Human	Gα-q and Gα-s	pcDNA3.1(-)	Klaus Groschner (Med.Univ. of Graz)
Muscarinic acetylcholine receptor M2 (M2R)	Human	Gα-i/o	pcDNA3.1(-)	MGC (MHS6278-211689788)
Muscarinic acetylcholine receptor M3 (M3R)	Human	Gα-q	endogenous	Atwood 2011

Table 4.2 List of control GPCRs used in agonist and light-activated chimeric forms form with their specie of provenience, expression vector and origin information

Only two full-length control GPCRs are endogenously expressed in HEK293 cells according to Atwood *et al.*[248], while all other full-length control GPCRs were transiently transfected using the mammalian expression pcDNA3.1(-), or equivalent mammalian expression vectors having a CMV promoter, in order to guarantee comparable expression efficiency (Table 4.2).

4.3 Validation of the luciferase-based reporter plasmids

I chose HEK293 cells to build the oGPCRs screening platform for the luciferase-based assays. According to Atwood *et al.*, HEK293 cells should theoretically express sufficient levels of G-Protein related proteins to activate G-Protein mediated intracellular cascades.

For each luciferase-based reporter plasmid I performed three different sets of experiments to validate the screening assay:

- I first agonist-stimulated HEK293 cells transfected with only the different luciferase-based reporter plasmids chosen for the screening of the G-

Protein mediated pathway and the Renilla plasmid. The set of agonists used was the same chosen for the stimulation of the ligand-activated control GPCRs. With this experiment I wanted to estimate efficiency of the reporter plasmids and the contribution of HEK293 cells endogenous GPCRs to background signals.

- Then, I stimulated HEK293 cells transiently transfected with the ligand-activated control GPCRs plus the Luciferase based reporter plasmid and the Renilla plasmid to estimate sensitivity and specificity of each reporter in recording increase in the Firefly/Renilla ratio due to agonist stimulation.

- Last, I light-stimulated the light-activated version of the control GPCRs to estimate the efficiency of the light stimulation experimental apparatus and the specificity and sensitivity of each reporter plasmid in recording light induced increase in the Firefly/Renilla ratios.

4.3.1 CRE reporter plasmid

I performed the validation of the CRE luciferase-based reporter plasmid performing the three different sets of experiments described in the previous section. The luciferase-based CRE reporter plasmid is supposed to be sensitive to rising in intracellular level of cAMP upon receptor activation. When not stated differently, HEK293 cells have always been transfected with the Renilla luciferase plasmid for all the experiments performed.

4.3.1.1 Agonist stimulation of endogenously expressed GPCRs

In a first set of experiments I stimulated endogenously expressed GPCRs in HEK293 cells transfected with the luciferase-based CRE reporter plasmid. I stimulated HEK293 cells using the set of agonists described in section 3.2.1 of the Material and Methods.

I performed these experiments to measure the response of HEK293 endogenously expressed GPCRs able to stimulate the CRE transcription factor.

I considered these responses as estimated background signals for the screening assay.

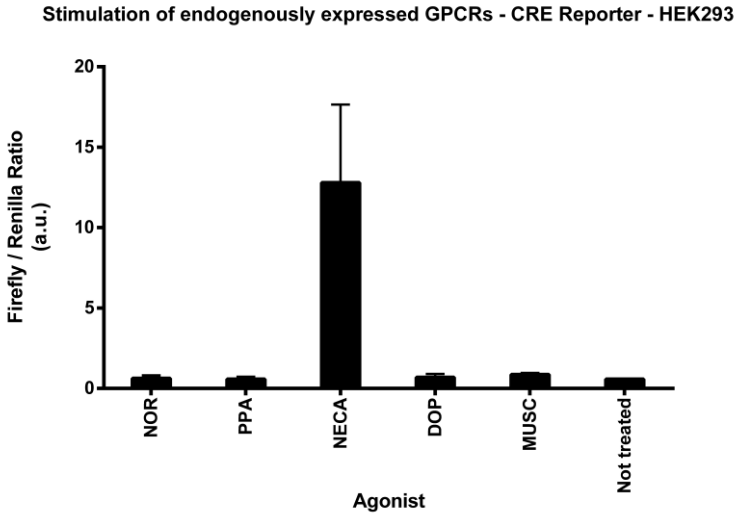

Figure 4.1 Agonist-stimulation of endogenously expressed GPCRs in HEK293 cells monitored using the luciferase-based CRE reporter plasmid

Only HEK293 cells stimulated with NECA showed a Firefly/Renilla ratio significantly higher in comparison with unstimulated cells. All other agonist shows Firefly/Renilla ratios not significantly higher of the ones recorded unstimulated cells.

The activation of the CRE reporter plasmid upon NECA stimulation is due to the stimulation of endogenously expressed GPCRs adenosine receptors A2A and A2B, as expected from literature.

Data represented in Figure 4.1 prove that:

- HEK293 cells levels of expression of G-Protein related proteins are sufficient to stimulate the CRE the reporter plasmid.
- The CRE reporter plasmid used is sensitive enough to detect signal coming from agonist stimulation of endogenously expressed GPCRs.
- The only significant signal background for the luciferase-based CRE reporter plasmid is due to NECA stimulation of endogenously expressed

adenosine receptors (A2A, A2B), while no significant background signal is recorded for any of the other agonist used.

4.3.1.2 Agonist stimulation of transiently transfected full-length control GPCRs

I agonist-stimulated HEK293 cells transiently transfected with the full-length control GPCRs listed in Table 4.2 in order to estimate the sensitivity and specificity of the luciferase-based CRE reporter plasmid in detecting signals coming from transiently transfected full-length control GPCRs.

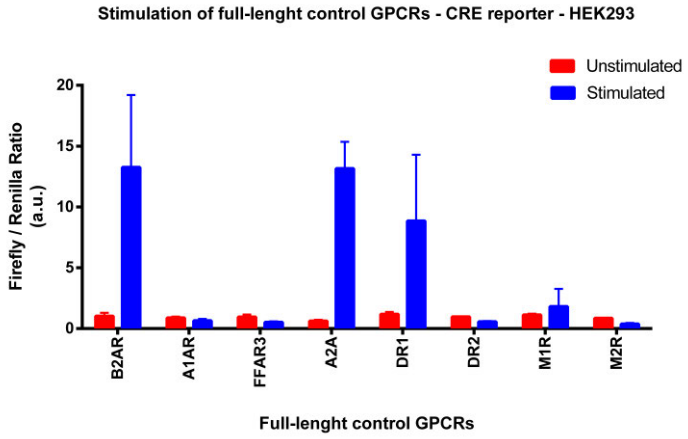

Figure 4.2 Agonist-stimulation of transiently transfected full-length control GPCRs in HEK293 cells monitored using the luciferase-based CRE reporter plasmid

HEK293 cells transiently transfected with full-length β2-adrenergic receptor (B2AR) and Dopamine 1 (DR1) show a significant increase in the Firefly/Renilla ratio upon Norepinephrine (B2AR) and Dopamine (DR1) stimulation. Considering the results in Figure 4.1, the signals recorded from Norepinephrine and Dopamine stimulation are dependent only on the activation of transiently transfected B2AR and DR1 and have are not dependent on endogenously expressed GPCRs. HEK293 cells stimulated with NECA showed an activation comparable with the one shown in Figure 4.1.

HEK293 cells transfected with Muscarinic 1 receptor (M1R) showed a weak increase in the Firefly/Renilla ratio upon stimulation with Muscarine. All other HEK293 cells transiently transfected with full-length control GPCRs showed no

significant increase in the Firefly/Renilla ratio upon agonist stimulation in comparison with unstimulated cells.

Data shown in Figure 4.2 prove that the luciferase-based CRE reporter plasmid is enough sensitive to discriminate signal coming from transiently transfected agonist stimulated full-length control GPCRs. Also, the CRE reporter plasmid is activated only through agonist stimulation of GPCRs (transiently transfected and endogenously expressed) for which ability to increase intracellular level of cAMP has been previously proved.

4.3.1.3 Light-stimulation of transiently transfected light-activated control GPCRs

I light-stimulated HEK293 cells transiently transfected with light-activated control GPCRs (Table 4.2) following the light-stimulation procedure described in section 3.3 of the Material and Methods.

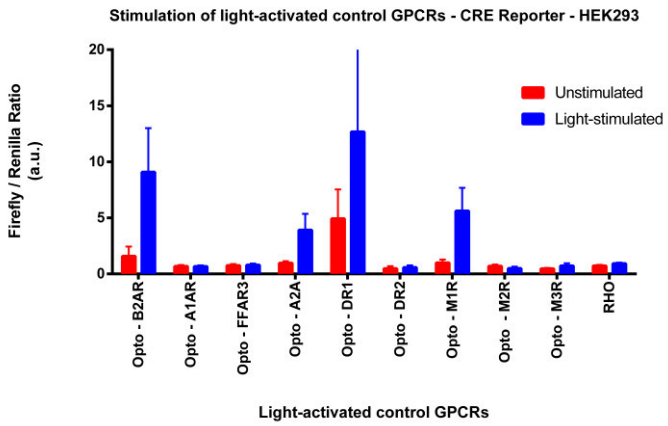

Figure 4.3 Light-stimulation of transiently transfected light-activated control GPCRs in HEK293 cells monitored using the luciferase-based CRE reporter plasmid

I considered the Firefly/Renilla ratio values in dark and light conditions for HEK293 cells transfected with rhodopsin ("RHO" column, Figure 4.3) as negative control, not expecting stimulation of the CRE transcription factor from the activation of Gα-t1/2.

Light-activated control GPCRs show an activation pattern comparable with the agonist stimulation of their respective full-length genes (Figure 4.3 vs Figure

4.2). Light-activated B2AR, DR1 and A2A showed a significant increase in the Firefly/Renilla ratio values upon light-stimulation.

Light-activated M1R present a light-dependent CRE stimulation significantly higher in comparison with the one recorded from the agonist stimulation of the full-length M1R (Figure 4.2).

I stimulated HEK293 cells transiently transfected with the full- length M1R with CCH (Carbachol, not selective agonist) and VU357017 (selective M1R agonist) in order to prove that the light induced response in the light-activated M1R is neither an artifact created by the assay or an artificial coupling created by the chimeric M1R. HEK293 cells transfected only the luciferase-based CRE reporter plasmid were as well stimulated using CCH and VU357017 and considered as negative control.

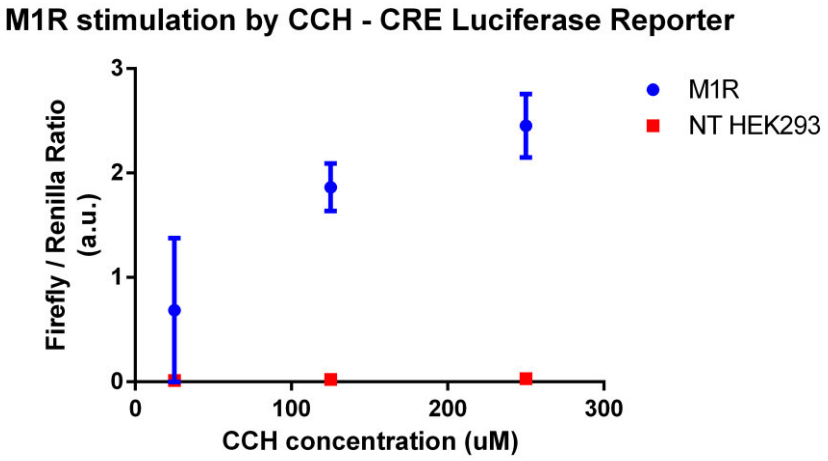

Figure 4.4 CCH stimulation of full-length M1R transiently transfected in HEK293 cells monitored using the luciferase-based CRE reporter plasmid

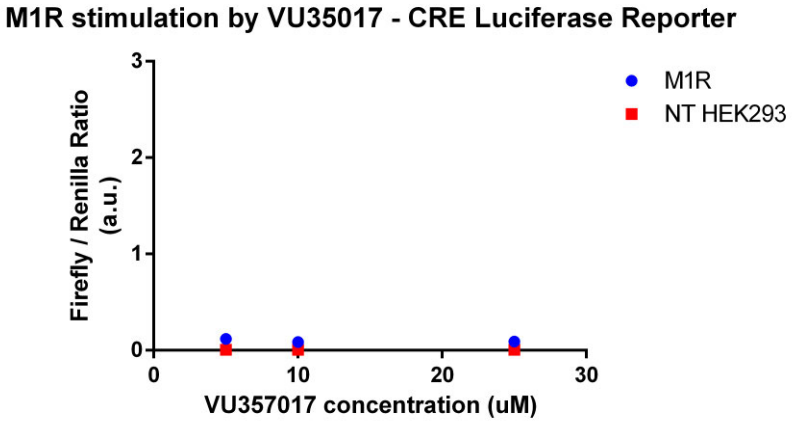

Figure 4.5 VU357017 stimulation of full-length M1R transiently transfected HEK293 cells monitored with the luciferase-based CRE reporter

HEK293 cells transiently transfected with the full-length M1R and stimulated with different concentrations of CCH shown Firefly/Renilla values significantly higher in comparison with HEK293 cells containing only the luciferase-based CRE reporter plasmid (Figure 4.4).

HEK293 cells transiently transfected with the full-length M1R and stimulated with different concentrations of VU35017 show no significant difference in the Firefly/Renilla ratio values in comparison with HEK293 cells only transfected with the luciferase-based CRE reporter plasmid (Figure 4.5).

The data represented in Figure 4.3, 4.4 and 4.5 suggest that the removal of the M1R ligand dependency in the light-activated M1R preserve the ability of the M1R intracellular loops to couple to Gα-s subunits, increase intracellular levels of cAMP and subsequently activate the CRE transcription factor.

Data shown in Figure 4.3 prove that:

- The light stimulation procedure described in section 3.3 is sufficient to stimulate light-activated control GPCRs.
- The CRE reporter plasmid is sensitive enough to record light-dependent CRE activation by transiently transfected light-activated control GPCRs.
- The light-activated control GPCRs showed a light-dependent CRE activation pattern (Figure 4.5) comparable with the activation pattern of their respective full-length genes.

4.3.2 SRE.L reporter plasmid

I performed the same three sets of experiments describe in section 3.1 to validate the luciferase-based SRE.L reporter plasmid. This reporter plasmid is supposed to be sensitive to RhoA activation. Except for when stated differently, in the following experiments HEK293 cells were always transfected with the Renilla luciferase plasmid.

4.3.2.1 Agonist stimulation of endogenously expressed GPCRs

I agonist-stimulated HEK293 cells transfected with the luciferase-based SRE.L reporter plasmid in order to measure activation coming from agonist stimulation

of endogenously expressed GPCRs and estimate background signal for this screening assay.

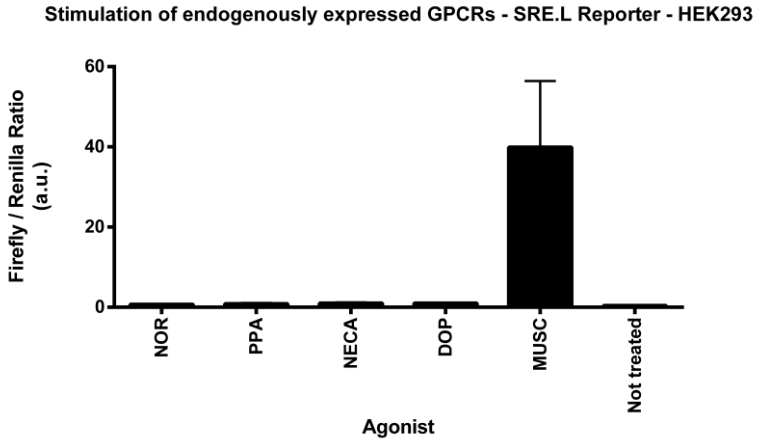

Figure 4.6 Agonist-stimulation of endogenously expressed GPCRs in HEK293 cells monitored using the luciferase-based CRE reporter plasmid

HEK293 cells stimulated with Muscarine show Firefly/Renilla ratio values significantly higher in comparison with same values measured for unstimulated cells, while all other agonists show values comparable with unstimulated cells.
According to the literature, HEK293 cells endogenously express a significant amount of Muscarine 3 receptor (M3R) supposed to be able to activate RhoA and consequently stimulate the SRE.L transcription factor.
Data shown in Figure 4.6 prove that the SRE.L reporter plasmid is sensitive enough to record signals coming from agonist stimulation of endogenously expressed GPCRs. Also, only Muscarine stimulated HEK293 cells show significant background signal coming from the activation of endogenously expressed M3R, while all other agonist shows comparable activation with unstimulated cells.

4.3.2.2 Agonist stimulation of transiently transfected full-length control GPCRs

I agonist-stimulated HEK293 cells transiently transfected with full-length control GPCRs and SRE.L reporter plasmid.

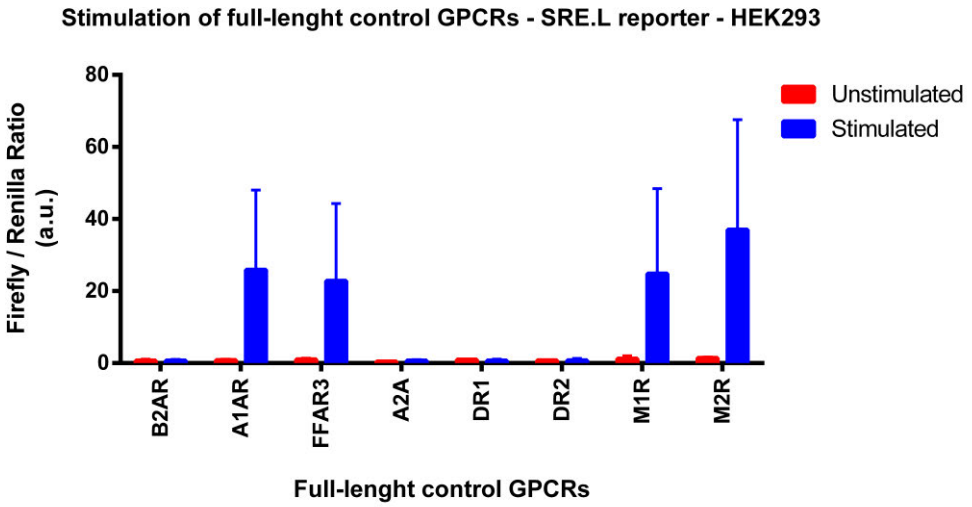

Figure 4.7 Agonist-stimulation of transiently transfected full-length control GPCRs in HEK293 monitored using the luciferase-based SRE.L reporter

HEK293 cells transiently transfected with α1-adrenergic receptor (A1AR), free-fatty acid 3 receptor (FFAR3), Muscarinic 1 (M1R) and Muscarinic 2 (M2R) show significant increase in Firefly/Renilla ratio values upon agonist stimulation. All other transiently transfected full-length controls show Firefly/Renilla ratio values comparable between stimulated and unstimulated states. While the induction recorded for the A1AR and FFAR3 can be exclusively related to the activation of the transiently transfected full-length control GPCRs, the M1R and M2R induction need further analysis. The M1R is known to be Gα-q coupled while the M2R is known to be Gα-i/o coupled. The data for agonist stimulation of HEK293 cells only SRE.L reporter transfected (Figure 4.6) show that Muscarine is able to activate the endogenously expressed M3R in HEK293 cells. This suggests that the M1R and M2R responses upon Muscarine stimulation (Figure 4.7) might not be dependent on the agonist stimulation of transiently transfected M1R and M2R, but on the endogenous response of M3R.

4.3.2.3 Light stimulation of transiently transfected light-activated control GPCRs

I light-stimulated HEK293 cells transfected with light-activated control GPCRs following the procedure explained in section 3.2.

No dark activity is recorded in HEK293 cells transiently transfected with light-activated control GPCRs. Firefly/Renilla values for rhodopsin are comparable under dark vs. light condition.

Light stimulation conserves the activation pattern of the agonist stimulation for the A1AR and FFAR3 receptors, while light-activated M1R and M2R shown no light induced activation of the SRE.L reporter.

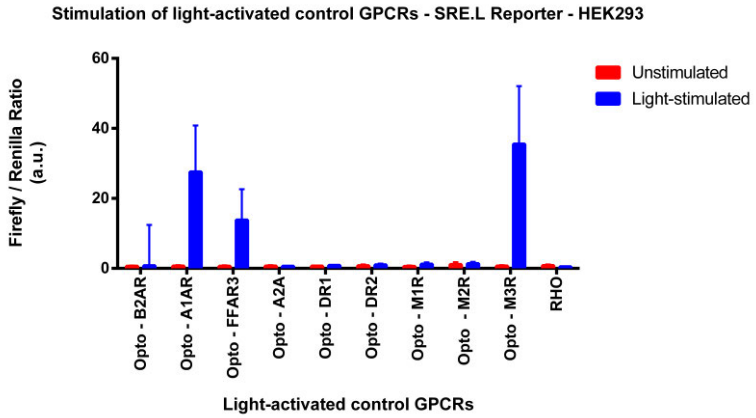

Figure 4.8 Light-stimulation of transiently transfected light-activated control GPCRs in HEK293 cells monitored using the luciferase-based SRE.L reporter plasmid

The absence of light-dependent stimulation of the SRE.L reporter plasmid by the light-activated M1R and M2R is in accordance with the hypothesis that the agonist dependent stimulation shown in Figure 4.6 for the M1R and M2R is due to the activation of endogenously expressed M3R, whose RhoA coupling and consecutive activation of the SRE.L responsive element is also confirmed by light activation of its corresponding light-activated chimera shown in Figure 4.10.

4.3.3 SRE reporter plasmid

To validated the luciferase-based SRE reporter plasmid I performed three sets of experiments described in section 4.1. The SRE reporter plasmid is supposed to be sensitive to MAPK and PKC activation. When not stated differently, HEK293 cells have to been considered always transfected with the Renilla plasmid.

4.3.3.1 Agonist stimulation of endogenously expressed GPCRs

I agonist-stimulated HEK293 cells transfected with the SRE reporter plasmid in order to record activity from stimulation of endogenously expressed GPCRs and estimate background signals for this reporter assay.

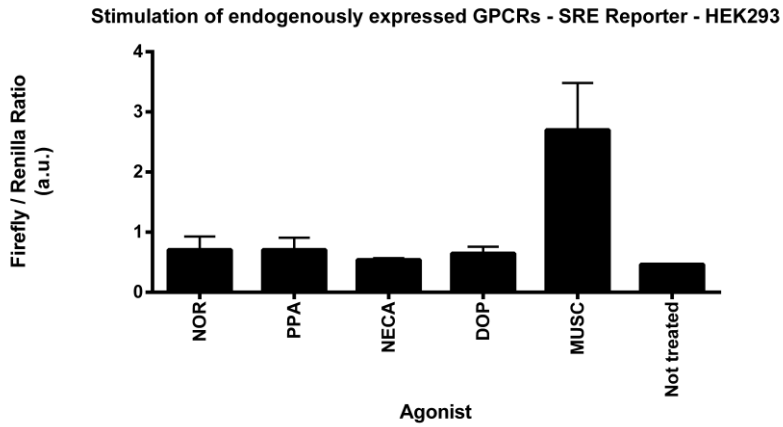

Figure 4.9 Agonist-stimulation of endogenously expressed GPCRs in HEK293 cells monitored using the luciferase-based SRE reporter

HEK293 cells stimulated with Muscarine show significant increase in the Firefly/Renilla ratio values in comparison with unstimulated cells, while all other agonist show Firefly/Renilla ratio values comparable with unstimulated cells.
The activation by Muscarine of SRE reporter plasmid can be explained by the activation of the endogenously expressed M3R, like already shown in Figure 4.6 for HEK293 cells transfected with the SRE.L reporter plasmid.

4.3.3.2 Agonist stimulation of transiently transfected full-length control GPCRs

I agonist stimulated HEK293 cells transiently transfected with full-length control GPCRs. Agonist-stimulated HEK293 cells transiently transfected with A1AR, FFAR3, M1R and M2R full-length control GPCRs show significant increase in the Firefly/Renilla ratio upon agonist stimulation in comparison with unstimulated cells. In the case of the A1AR and FFAR3 the increase in the Firefly/Renilla ratio values in the stimulated states is due only to the activation of the transiently transfected full-length genes.

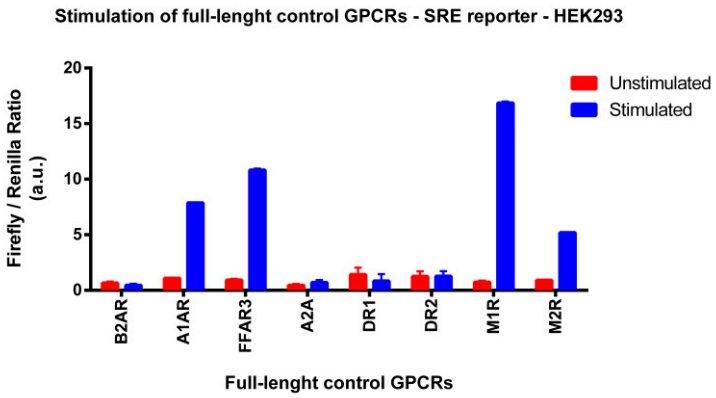

Figure 4.10 Agonist-stimulation of transiently transfected full-length control GPCRs in HEK293 cells monitored using the luciferase-based SRE reporter plasmid

The response of the M1R can be justified by agonist stimulation of the transiently transfected M1R receptor and the endogenously expressed M3R, while the response of the M2R receptor is entirely derived from the agonist stimulation of endogenously expressed M3R, as supported also by the results of the equivalent experiment performed using the SRE.L reporter plasmid (Figure 4.7)

4.3.3.3 Light stimulation of transiently transfected light-activated control GPCRs

I light-stimulated HEK293 cells transiently transfected with light-activated control GPCRs following the procedure described in section 3.1 of the Material and Methods.

Light-stimulated HEK293 cells transiently transfected with light-stimulated control GPCRs shown an activation pattern (Figure 4.11) comparable with the agonist-induced activation pattern of the respective full-length control genes (Figure 4.10) in the case of the A1AR, FFAR3 and M1R receptors.

The absence of signal from the light-activated M2R control GPCRs validate as M3R dependent the ligand induced activation shown in Figure 4.10. The agonist-induced response for cells transiently transfected with the M1R (Figure 4.10) can be considered as the sum of the endogenous stimulation of the M3R plus the stimulation of the transiently transfected M1R.

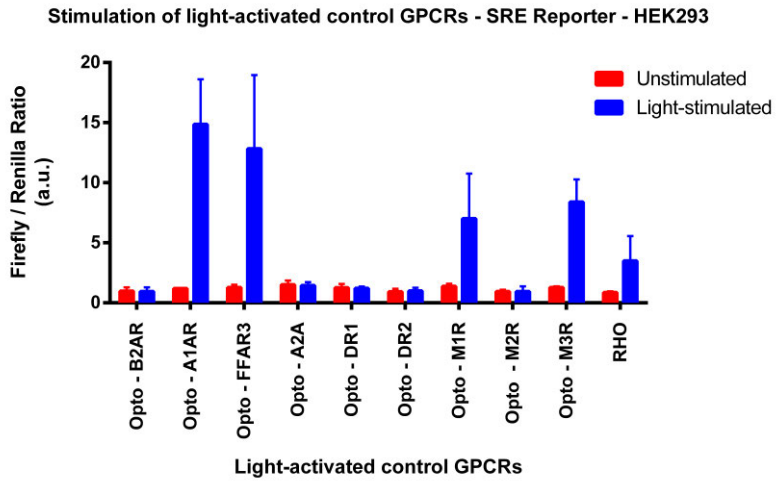

Figure 4.11 Light-stimulation of transiently transfected light-activated control GPCRs monitored using the luciferase-based SRE reporter

Figure 4.11 and 4.10 show that the SRE Luciferase reporter plasmid can be used to screen PKC and MAPK dependent G-Protein mediated pathways in the functional assay for the light-activated oGPCRs.

4.3.4 CRE reporter plasmid to monitor cAMP depletion

The luciferase-based CRE reporter plasmid can be used to screen for reduction of intracellular level of cAMP upon receptor activation. HEK293 cells can be pre-stimulated in order to increase intracellular levels of cAMP.

In theory pre-stimulated cells expressing receptor known to be Gα-i/o couple will have at the end of the screening assay lower Firefly/Renilla ratio values in comparison with the other pre-stimulated cells.

In order to verify this hypothesis, I performed the three sets of experiments already performed for the other luciferase-based reporter plasmids, plus an additional experiment to estimate the right concentration of agonist to use for HEK293 pre-stimulation.

4.3.4.1 NECA dose response curve for pre-stimulation of HEK293 cells

As already shown for the validation of the luciferase-based CRE reporter plasmid HEK293 cells endogenously express a sufficient amount of adenosine receptors to stimulate production of cAMP upon NECA stimulation.

In a first set of experiments I tested different concentrations of NECA to stimulate endogenously expressed adenosine receptors A2A and A2B in HEK293 cells to increase the amount of intracellular cAMP levels.

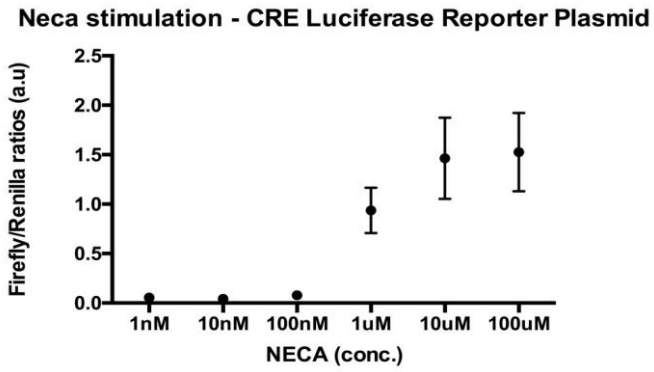

Figure 4.12 NECA stimulation (at different concentrations) of HEK293 cells monitored using the luciferase- based CRE reporter

Using different concentrations of NECA I was able to calculate a dose response curve for stimulation of endogenously expressed GPCRs in HEK293 cells transfected with the luciferase-based CRE reporter plasmid.
I chose 500nM as final concentration for HEK293 pre-stimulation with NECA considering this values as close to the NECA EC50.

4.3.4.2 Agonist stimulation of endogenously expressed GPCRs

I pre-stimulated HEK293 cells transfected with the luciferase-based CRE reporter plasmid with 500nm of NECA for five minutes (see section 3.2) prior agonist stimulation (following the same procedure used for previous luciferase- based reporter plasmids validations).
In this assay the values of the Firefly/Renilla ratios for control GPCRs that are known to decrease intracellular cAMP levels by coupling with Gα-i/o subunits are supposed to be lower in the case of the ligand/light stimulated state in comparison with the unstimulated one.

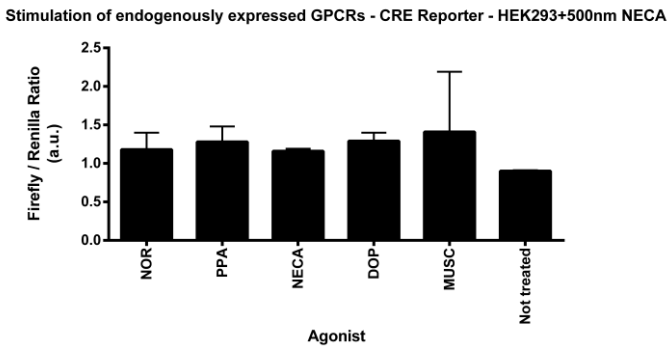

Figure 4.13 Agonist-stimulation of NECA prestimulated HEK293 cells monitored using the luciferase-based CRE reporter plasmid

All agonist stimulated HEK293 cells show Firefly/Renilla ratio values comparable with the values of HEK293 cells only pre-stimulated with 500 nm of NECA (Figure 4.15). No further CRE stimulation is recorded for any of the agonist-stimulated HEK293 cells, suggesting that HEK293 do not endogenously express sufficient levels of any Gα-i/o coupled GPCRs.

4.3.4.3 Agonist stimulation of transiently transfected full-length control GPCRs

I agonist-stimulated HEK293 transiently transfected with full-length control GPCRs after pre-stimulation with 500nM of NECA.

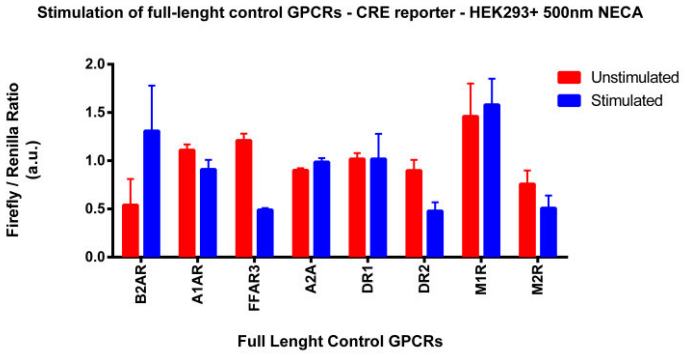

Figure 4.14 Agonist-stimulation of transiently transfected full-length control GPCRs in prestimulated HEK293 cells monitored using the luciferase-based CRE reporter plasmid

FFAR3, DR2 and M2R show lower values of Firefly/Renilla ratios in the agonist-stimulated state in comparison with the unstimulated state. These receptors tare known by literature to be able to lower the amount of intracellular cAMP levels upon agonist stimulation. The FFAR3 receptor shows a strong reduction in cAMP levels that is in accordance with previously published work. All other control receptors show Firefly/Renilla ratio values comparable with the values of NECA pre-stimulated HEK293 cells.

Data shown in Figure 4.14 suggest that NECA pre-stimulation of HEK293 cells transfected with the luciferase-based CRE reporter plasmid is a valid approach to screen for decrease of intracellular level of cAMP upon agonist-stimulation of transiently transfected control GPCRs.

4.3.3.4 Light stimulation of transiently transfected light-activated control GPCRs

I light stimulated pre-stimulated HEK293 cells transiently transfected with light-activated control GPCRs.

The light-activated control GPCRs show a light-activation pattern in accordance to their respective ligand-activated receptors (Figure 4.14 vs. Figure 4.15)

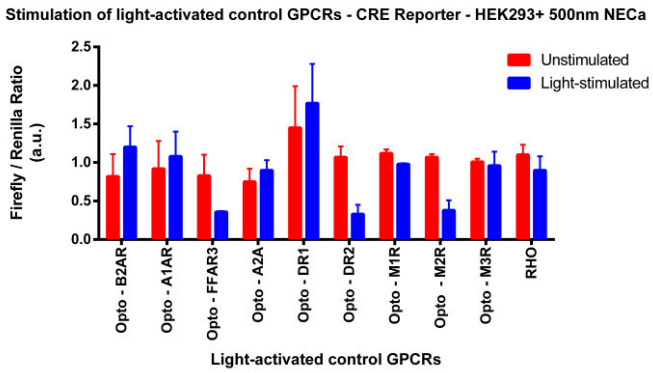

Figure 4.15 Light-stimulation of transiently transfected light-activated control GPCRs in prestimulated HEK293 cells using the luciferase-based CRE reporter

Light-activated FFAR3, D2R and M2R show a significant reduction in Firefly/Renilla ratios in the light-activated states in comparison with dark values,

while all other light-activated control GPCRs show Firefly/Renilla ratio values comparable for the in the dark and light state.

The data presented in Figure 4.14 and 4.15 show that is possible to record reduction of intracellular level of cAMP upon light-stimulation of light-sensitive control GPCRs Gα-i/o coupled using HEK293 cells transfected with the luciferase-based CRE reporter plasmid and pre-stimulated with 500nM of NECA.

4.4 Functional screening of light-activated human Class A oGPCRs

The luciferase based reporter plasmid previously described for which I performed a full validation using both transiently transfected full-length and light-activated control GPCRs were used to set up the screening platform for all the selected 64 light-activated human Class A oGPCRs using HEK293 cells. The screening platform and the data analysis were set up following the procedure described in sections 4.2 and 4.3 of the Material and Methods. Only when not stated differently, HEK293 cells were always transfected with the Renilla plasmid for all the following described experiments.

4.4.1 CRE reporter

I transfected HEK293 cells with all 64 light-activated human Class A oGPCRs, CRE reporter. HEK293 cells were light stimulated according to procedure described in the section 3.1.1.

None of the 64 light-activated human Class A oGPCRs screened used the luciferase-based CRE reporter plasmid shows significant activity in the dark, with Firefly/Renilla ratios comparable to the ratios under dark condition of light-activated control receptors and rhodopsin shown in Figure 4.1, 4.2 and 4.3.

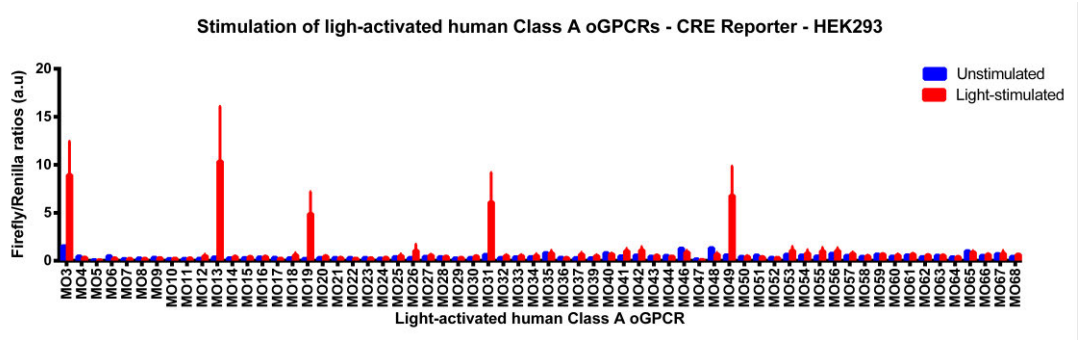

Figure 4.16 Light-stimulation of transiently transfected light-activated human Class A oGPCRs in HEK293 cells monitored using the luciferase-based CRE reporter

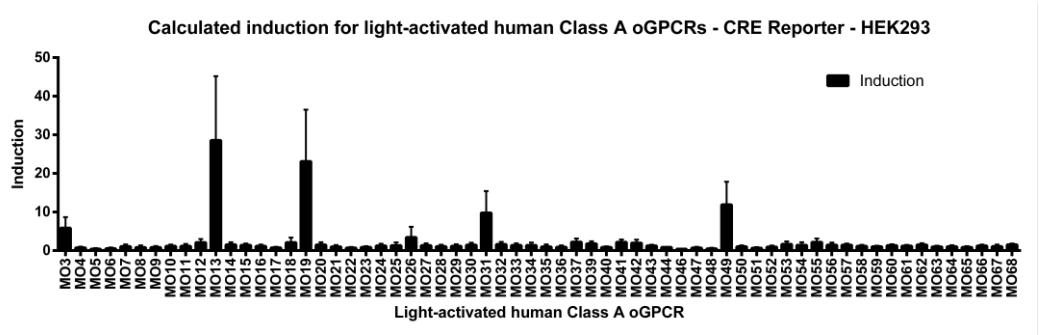

Figure 4.17 Induction values for light-stimulated human Class A oGPCRs calculated on the data collected using the luciferase-based CRE reporter

I calculated induction values from the data represented in Figure 4.16 (Figure 4.17) for all 64 light-activated human Class A oGPCRs by dividing Firefly/Renilla values after light stimulation with dark values. I set as threshold to identify strong activation from light-activated human Class A oGPCRs an induction value higher or equal to 2.5 (that implies more than a two-fold increase in the Firefly/Renilla ratio between light and dark conditions).

Six light-activated Class A oGPCRs show an induction value above the defined threshold:

Identifier	Gene Name	Induction value
MO3	Opto-**GPR1**	5.87
MO13	Opto-**GPR21**	28.55
MO19	Opto-**GPR32**	23.07
MO26	Opto-**GPR42**	3.45
MO31	Opto-**GPR61**	9.78

MO49	Opto-**GPR135**	11.89

Table 4.8 Orphan genes for which significant light-dependent induction was measured using the luciferase-based CRE reporter

4.4.2 SRE.L reporter

I light-stimulated HEK293 cells transiently transfected with all 64 light-activated human Class A oGPCRs and the luciferase-based SRE.L reporter plasmid.

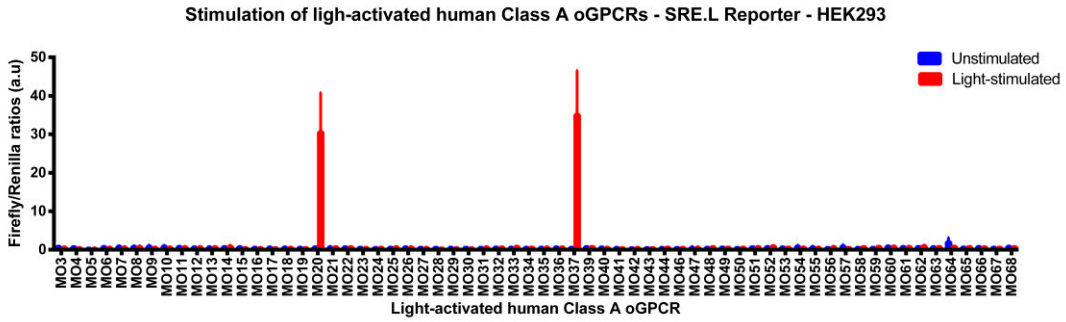

Figure 4.18 Light-stimulation of transiently transfected light-activated human Class A oGPCRs in HEK293 cells monitored using the luciferase-based SRE.L reporter

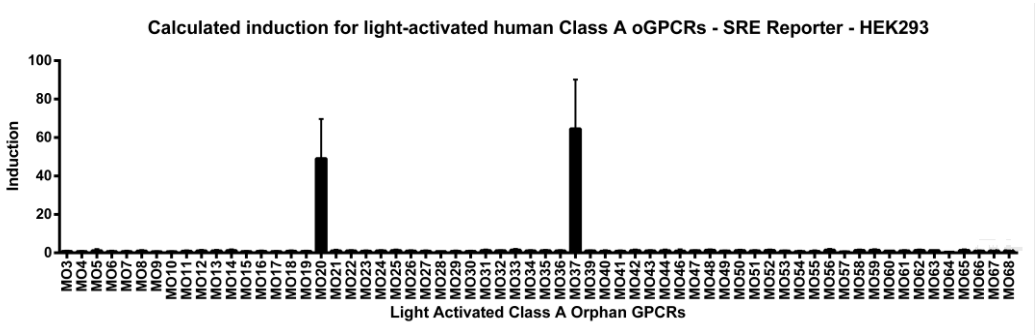

Figure 4.19 Induction values for light-stimulated human Class A oGPCRs calculated on the data collected using the luciferase-based SRE.L reporter

No light-activated human Class A oGPCRs shows significant activity in the dark, with Firefly/Renilla ratios comparable to the ratios under dark condition of light - activated control receptors and rhodopsin shown in Figure 4.7 and Figure 4.8.
I calculated induction values from the data represented in Figure 4.20 for all 64 light-activated human Class A oGPCRs. I set as threshold to identify strong

activation from light-activated human Class A oGPCRs an induction value higher or equal to 2.5

Two light-activated human Class A oGPCRs show an induction value above the defined threshold, while the remaining 62 receptors do not show any significant light dependent activation:

Identifier	Gene Name	Induction value
MO20	Opto-**GPR33**	49.1
MO37	Opto-**GPR78**	64.5

Table 4.9 Orphan genes for which significant light dependent induction was measured in SRE.L reporter based screening assays

4.4.3 SRE reporter

I transfected HEK293 cells with all 64 light-activated human Class A oGPCRs, SRE reporter and Renilla luciferase plasmid. Cells were light stimulated following the procedure describe in section 3.2.1.

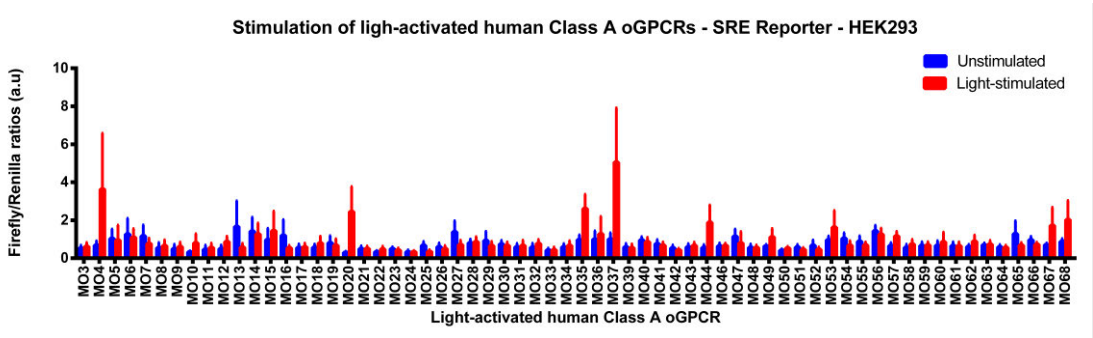

Figure 4.20 Light-stimulation of transiently transfected light-activated human Class A oGPCRs in HEK293 cells monitored using the luciferase-based SRE reporter

No light-activated chimeric orphan receptor shows significant activity in the dark, with Firefly/Renilla ratios comparable to the ratios under dark condition of light-activated control receptors and rhodopsin as shown in Figures 4.14 and 4.15 (Figure 4.20)

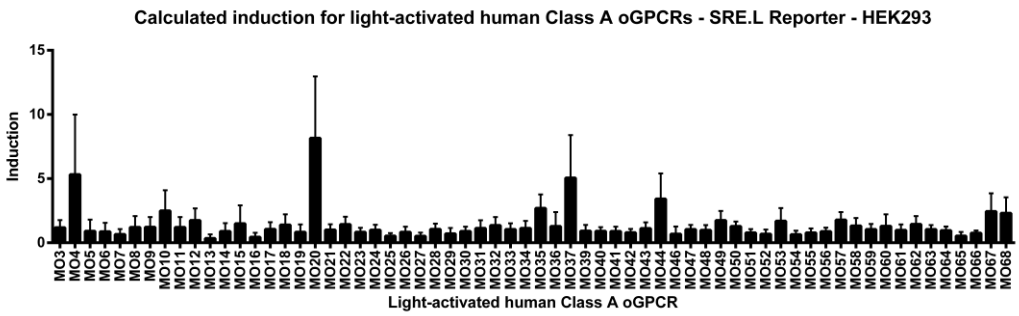

Figure 4.21 Induction values for light-stimulated human Class A oGPCRs calculated on the data collected using the luciferase-based SRE.L reporter

I calculated induction values from the data represented in Figure 4.20 (Figure 4.21) for all 64 light-activated chimeric Class A oGPCRs. I set as threshold to identify strong activation from light-activated Class A oGPCRs an induction value higher or equal to 2.5

Six light-activated human Class A oGPCRs show an induction value above the fixed threshold, while all remaining 58 GPCRs do not show any significant light dependent activation:

Identifier	Gene Name	Induction value
MO4	Opto-**GPR3**	5.32
MO10	Opto-**GPR18**	2.49
MO20	Opto-**GPR33**	2.69
MO35	Opto-**GPR68**	3.45
MO37	Opto-**GPR78**	5.05
MO44	Opto-**GPR88**	3.41

Table 4.10 Orphan genes for which significant light dependent induction was measured in SRE reporter based screening assays

4.4.4 CRE reporter plasmid and NECA prestimulation

I transfected HEK293 cells with all 64 light-activated human Class A oGPCRs. Cells were prestimulated with 500nm of NECA and then light-stimulated according to the procedure describe in section 3.2.1.

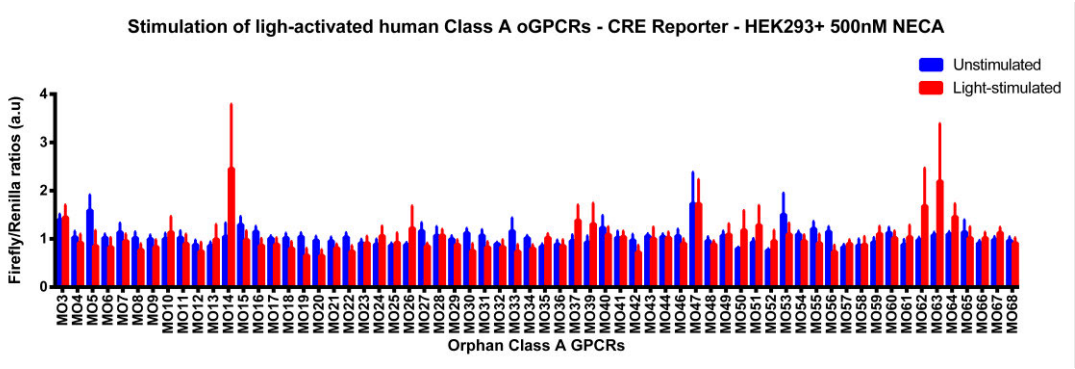

Figure 4.22 Light-stimulation of transiently transfected light-activated human Class A oGPCRs in prestimulated HEK293 cells monitored using the luciferase-based CRE reporter

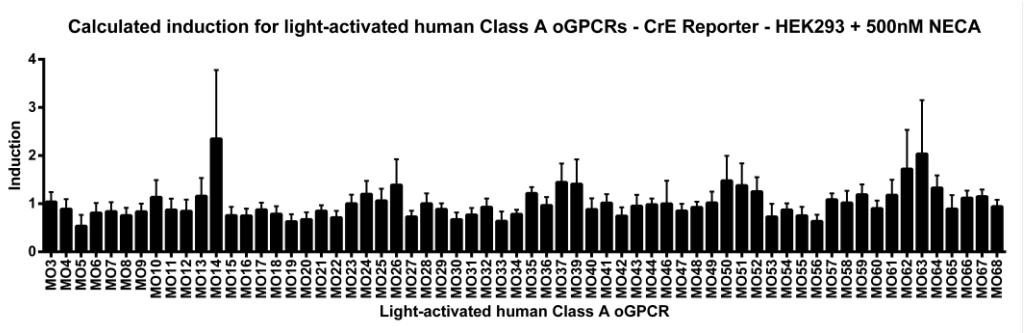

Figure 4.23 Induction values for light-stimulated human Class A oGPCRs calculated on the data collected using the luciferase-based CRE reporter after HEK293 NECA prestimulation

I calculated induction values from the data represented in Figure 4.22 for all 64 light-activated chimeric Class A oGPCRs (Figure 4.23). For this functional screening it is not possible to use the same induction threshold used for the previously described reporter plasmids. In this experimental setup, using the CRE reporter plasmid with NECA pre-stimulation, I expect a reduction in the Firefly/Renilla ratio values under light conditions in comparison with the dark values. I defined as new threshold a value of “reverse” induction (still calculated as Firefly/Renilla ratio value under light conditions divided for the same ratio under dark conditions) 0.7, which implies a minimum of 30% reduction between light and dark Firefly/Renilla ratios.

Six light-activated human Class A oGPCR show an induction value below the fixed threshold, while all the others 58 GPCRs do not show any significant light dependent decrease for intracellular cAMP levels:

Identifier	Gene Name	Induction value
MO5	Opto-**GPR4**	0.53
MO19	Opto-**GPR32**	0.63
MO20	Opto-**GPR33**	0.67
MO30	Opto-**GPR55**	0.67
MO33	Opto-**GPR63**	0.63
MO56	Opto-**GPR150**	0.64

Table 3.11 Orphan genes for which significant light dependent induction was measured in NECA prestimulated CRE reporter based screening assays

4.5 Antibody staining of all 64 light-activate human Class A oGPCRs

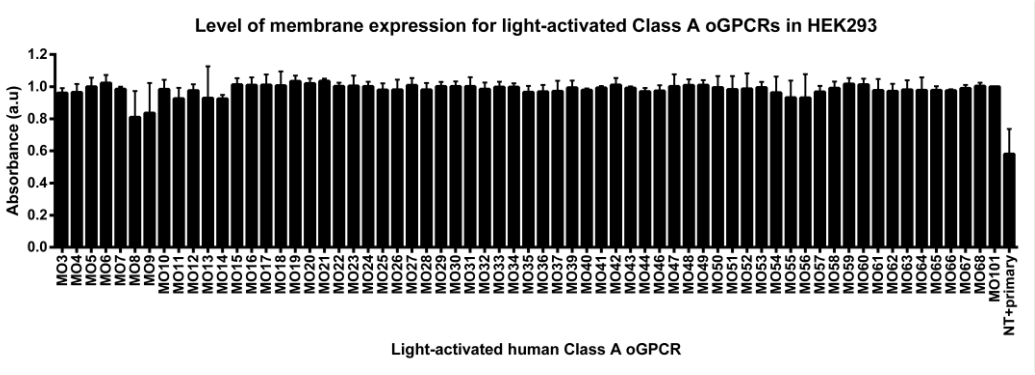

Figure 4.24 Absorbance normalized by the value of Rhodopsin (MO101) for the 64 light-activated Class A oGPCRs

I transfected HEK293 cells with all 64 light-activated human Class A oGPCRs and bovine rhodopsin. Cells were light stimulated and later stained with an anti-VSVG antibody (see section 3.1) following the procedure described in section 3.3.

Absorbance values normalized for Rhodopsin (indicated with MO101 in Figure 4.24) for all 64 light-activated human Class A oGPCRs show significant transient expression in light stimulated HEK293 cells The fixation technique used allows me to consider the absorbance values show in figure 4.24 for all 64 light-activated human Class A oGPCRs has to be related mostly to expression of the light-activated human Class A oGPCRs on the cell membrane.

All 64 light-activated human chimeric human Class A oGPCRs show significant level of membrane expression in comparison with the positive control

rhodopsin, with the exception of Opto-GPR12 and Opto-GPR15 that show a lower but yet still significant expression level in comparison with expression level of rhodopsin and the negative control.

4.6 Validation of G-Protein coupling prediction algorithms

I used the PREDCOUPLE, PREDCOUPLE2 and GRIFFIN prediction algorithms to study in silico the predicted coupling of 64 light-activated human Class A oGPCRs chosen for this study, their corresponding complete orphan sequences and ligand and light-activated control GPCRs.

The first criterion I adopted to validate each single algorithm was the agreement between the predicted coupling for the control GPCRs and the coupling reporter by literature and the experiment I performed using Luciferase based reporter plasmids.

Secondly, I considered the agreement between the predicted couplings for the full-length 64 human Class A oGPCRs I selected for this work and their respective light-activated chimeric genes. According to the hypothesis on which my screening using chimeric receptor is based and according to the data shown for control GPCRs, the coupling between full-length GPCRs and their respective chimeric receptor has to be the same.

As third criterion I consider the overall agreement between the three different algorithms I used to study the coupling of orphan and control GPCRs. Although based on different mathematical and statistical approaches, the outcome for the predicted coupling for control GPCRs and oGPCRs in both their ligand and light-activated form should be algorithm independent.

4.6.1 Validation of the GRIFFIN algorithm

4.6.1.1 Full-length control GPCRs

I run the Griffin algorithm with the sequence of the full-length control GPCRs described in table 4.6 in order to estimate the algorithm performance with GPCRs for which coupling properties are already known.

Control Receptor	Predominant G. coupling(experimental)	Predicted G. Coupling
β2 adrenergic receptor (β2AR)	Gα-s	Gα-s
α1 adrenergic receptor (α1AR)	Gα-q	Gα-q
Free fatty acid receptor 3 (FFR3)	Gα-i/o	Gα-i/o
Adenosine A2A receptor (A2AR)	Gα-s	Gα-s
Dopamine receptor D1 (D1R)	Gα-s	Gα-s
Dopamine receptor D2 (D2R)	Gα-i/o	Gα-i/o
Muscarinic acetylcholine receptor M1 (M1R)	Gα-q and Gα-s	Gα-q and Gα-s
Muscarinic acetylcholine receptor M2 (M2R)	Gα-i/o	Gα-i/o
Muscarinic acetylcholine receptor M3 (M3R)	Gα-q	Gα-q

Table 4.12 Predicted G-Protein coupling for full-length control GPCRs according to the GRIFFIN algorithm

The Griffin algorithm scored a 9/9 of correct predictions when analyzing sequences of full-length control GPCRs, showing that is able to predict correct coupling when analyzing entire sequences of GPCRs genes.

4.6.1.2 Light-activated control GPCRs

I run the GRIFFIN algorithm on the sequences of the light-activated control GPCRs to check if the algorithm is able to predict the correct coupling of chimeric GPCRs for which I have proved preservation of coupling properties in the experiment described in section 4.3.1.3, 4.3.2.3, 4.3.3.4.

Light-activated control Receptor	Predominant G coupling (experimental)	Predicted G. Coupling
β2 adrenergic receptor (β2AR)	Gα-s	Gα-t1/2
α1 adrenergic receptor (α1AR)	Gα-q	Gα-t1/2
Free fatty acid receptor 3 (FFR3)	Gα-i/o	Gα-t1/2

Adenosine A2A receptor (A2AR)	Gα-s	Gα-t1/2
Dopamine receptor D1 (D1R)	Gα-s	Gα-t1/2
Dopamine receptor D2 (D2R)	Gα-i/o	Gα-t1/2
Muscarinic acetylcholine receptor M1 (M1R)	Gα-q and Gα-s	Gα-t1/2
Muscarinic acetylcholine receptor M2 (M2R)	Gα-i/o	Gα-t1/2
Muscarinic acetylcholine receptor M3 (M3R)	Gα-q	Gα-t1/2

Table 4.13 Predicted G-Protein coupling for full-length control GPCRs according to the GRIFFIN algorithm

The Griffin algorithm scored a 0/9 of positive result when predicting G-Protein coupling for light-activated control GPCRs. All light-activated control GPCRS are predicted to be coupled to Gα-t1/2 that is the G-Protein mediated pathway activated by rhodopsin.

This result suggests that the GRIFFIN algorithm is not able to distinguish between bovine rhodopsin and chimeric receptor for which bovine rhodopsin constitute the majority of their sequences.

4.6.1.3 Human Class A oGPCRs

I run the GRIFFIN algorithm on all 64 Human Class A oGPCRs both on their full sequences and in their respective light-activated version.

Gene name	Identifier	Full-length predicted coupling	Light-activated predicted coupling
GPR1	**MO3**	Gα-q	Gα-t1/2
GPR3	**MO4**	Gα-s	Gα-t1/2
GPR4	**MO5**	Gα-q	Gα-t1/2
GPR6	**MO6**	Gα-s	Gα-t1/2
GPR12	**MO7**	Gα-s	Gα-t1/2
GPR15	**MO8**	Gα-q	Gα-t1/2
GPR17	**MO9**	Gα-q	Gα-t1/2

GPR18	**MO10**	Gα-q	Gα-t1/2
GPR19	**MO11**	Gα-i/o	Gα-t1/2
GPR20	**MO12**	Gα-q	Gα-t1/2
GPR21	**MO13**	Gα-i/o	Gα-t1/2
GPR22	**MO14**	Gα-i/o	Gα-t1/2
GPR25	**MO15**	Gα-q	Gα-t1/2
GPR26	**MO16**	Gα-i/o	Gα-t1/2
GPR27	**MO17**	Gα-q	Gα-t1/2
GPR31	**MO18**	Gα-i/o	Gα-t1/2
GPR32	**MO19**	Gα-s	Gα-t1/2
GPR33	**MO20**	Gα-q	Gα-t1/2
GPR34	**MO21**	Gα-s	Gα-t1/2
GPR35	**MO22**	Gα-s	Gα-t1/2
GPR37	**MO23**	Gα-q	Gα-t1/2
GPR37L1	**MO24**	Gα-s	Gα-t1/2
GPR39	**MO25**	Gα-q	Gα-t1/2
GPR42	**MO26**	Gα-q	Gα-t1/2
GPR45	**MO27**	Gα-i/o	Gα-t1/2
GPR50	**MO28**	Gα-s	Gα-t1/2
GPR52	**MO29**	Gα-i/o	Gα-t1/2
GPR55	**MO30**	Gα-q	Gα-t1/2
GPR61	**MO31**	Gα-s	Gα-t1/2
GPR62	**MO32**	Gα-i/o	Gα-t1/2
GPR63	**MO33**	Gα-i/o	Gα-t1/2
GPR65	**MO34**	Gα-q	Gα-t1/2
GPR68	**MO35**	Gα-q	Gα-t1/2
GPR75	**MO36**	Gα-s	Gα-t1/2
GPR78	**MO37**	Gα-i/o	Gα-t1/2
GPR82	**MO39**	Gα-q	Gα-t1/2
GPR83	**MO40**	Gα-q	Gα-t1/2
GPR84	**MO41**	Gα-s	Gα-t1/2

GPR85	**MO42**	Gα-q	Gα-t1/2
GPR87	**MO43**	Gα-q	Gα-t1/2
GPR88	**MO44**	Gα-q	Gα-t1/2
GPR119	**MO46**	Gα-s	Gα-t1/2
GPR120	**MO47**	Gα-q	Gα-t1/2
GPR132	**MO48**	Gα-i/o	Gα-t1/2
GPR135	**MO49**	Gα-i/o	Gα-t1/2
GPR139	**MO50**	Gα-s	Gα-t1/2
GPR141	**MO51**	Gα-s	Gα-t1/2
GPR142	**MO52**	Gα-s	Gα-t1/2
GPR146	**MO53**	Gα-s	Gα-t1/2
GPR148	**MO54**	Gα-s	Gα-t1/2
GPR149	**MO55**	Gα-s	Gα-t1/2
GPR150	**MO56**	Gα-s	Gα-t1/2
GPR151	**MO57**	Gα-s	Gα-t1/2
GPR152	**MO58**	Gα-s	Gα-t1/2
GPR153	**MO59**	Gα-q	Gα-t1/2
GPR160	**MO60**	Gα-s	Gα-t1/2
GPR161	**MO61**	Gα-s	Gα-t1/2
GPR162	**MO62**	Gα-q	Gα-t1/2
GPR171	**MO63**	Gα-q	Gα-t1/2
GPR173	**MO64**	Gα-s	Gα-t1/2
GPR174	**MO65**	Gα-s	Gα-t1/2
GPR176	**MO66**	Gα-s	Gα-t1/2
GPR182	**MO67**	Gα-q	Gα-t1/2
GPR183	**MO68**	Gα-q	Gα-t1/2

Table 4.14 Coupling prediction for 64 human Class A oGPCRs in their full-length and light-activated forms according to the GRIFFIN algorithm

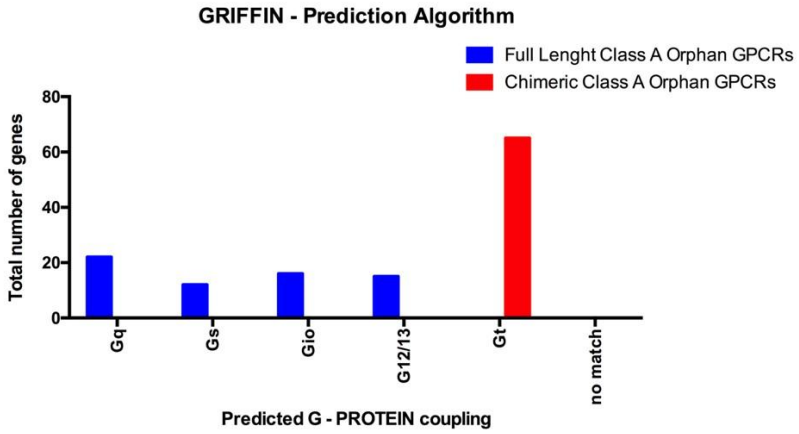

Figure 4.25 Predicted G-Protein coupling for 64 full-length and relative light-activated human Class A oGPCRs according to the Griffin Algorithm.

The GRIFFIN algorithm is able to predict a possible coupling for all 64 full- length human Class A oGPCRs sequences, while all respective light-activated version is predicted to be Gα-t1/2 coupled, as already shown in the previous section for light-activated control GPCRs.

This result confirm that the GRIFFIN algorithm is not sensitive enough to predict a reasonable coupling for light-activated chimeric receptor for which has been shown conservation of coupling properties (like in the case of light-activated control GPCRs) and to distinguish between rhodopsin based chimeric receptor and rhodopsin itself.

4.6.2 Validation of the PREDCOUPLE algorithm

4.6.2.1 Full-length control GPCRs

I run the PREDCOUPLE algorithm with the sequences of the full-length control GPCRs described in Table 3.6 in order to estimate the algorithm performance with full-length GPCRs for which coupling properties are already known.

Control Receptor	Predominant G-coupling (Experimental)	Predicted G-Coupling
β2 adrenergic receptor (β2AR)	Gα-s	Gα-s
α1 adrenergic receptor (α1AR)	Gα-q	Gα-q
Free fatty acid receptor 3 (FFR3)	Gα-i/o	Gα-i/o
Adenosine A2A receptor	Gα-s	Gα-s

(A2AR)		
Dopamine receptor D1 (D1R)	Gα-s	Gα-s
Dopamine receptor D2 (D2R)	Gα-i/o	Gα-i/o
Muscarinic acetylcholine receptor M1 (M1R)	Gα-q and Gα-s	Gα-q
Muscarinic acetylcholine receptor M2 (M2R)	Gα-i/o	Gα-i/o
Muscarinic acetylcholine receptor M3 (M3R)	Gα-q	Gα-q

Table 4.14 Predicted G-Protein coupling for full-length control GPCRs according to the PREDCOUPLE algorithm

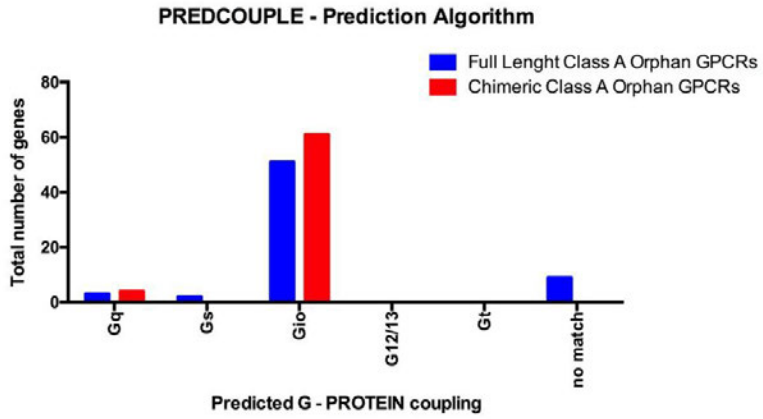

Figure 4.26 Predicted G-Protein coupling for 64 full-length and relative light-activated human Class A oGPCRs according to the PREDCOUPLE Algorithm.

The PREDCOUPLE algorithm scored a 9/9 of correct predictions when analyzing sequences of full-length control GPCRs, showing that is able to predict correct coupling when analyzing entire sequences of GPCRs genes.

4.6.2.2 Light-activated control GPCRs

I run the PREDCOUPLE algorithm on the sequences of the light-activated control GPCRs to check if the algorithm is able to predict the correct coupling of chimeric GPCRs for which I have proved preservation of coupling properties in the experiment described in section 4.3

Light-activated control Receptor	Predominant G coupling (experimental)	Predicted G. Coupling
β2 adrenergic receptor (β2AR)	Gα-s	Gα-s

α1 adrenergic receptor (α1AR)	Gα q	Gα-q
Free fatty acid receptor 3 (FFR3)	Gα-i/o	Gα-i/o
Adenosine A2A receptor (A2AR)	Gα-s	Gα-s
Dopamine receptor D1 (D1R)	Gαs	Gα-s
Dopamine receptor D2 (D2R)	Gα-i/o	Gα-i/o
Muscarinic acetylcholine receptor M1 (M1R)	Gα-q and Gα-s	Gα-q
Muscarinic acetylcholine receptor M2 (M2R)	Gα-i/o	Gα-i/o
Muscarinic acetylcholine receptor M3 (M3R)	Gα-q	Gα-q

Table 4.15 Predicted G-Protein coupling for full-length control GPCRs according to the PREDCOUPLE algorithm

The PREDCOUPLE algorithm scored a 9/9 of positive result when predicting G-Protein coupling for light-activated control GPCRs. All light-activated control GPCRS are predicted to be coupled equivalently to their full-length respective genes and in accordance to what I proved with the experiment described in section

This result prove that the PREDCOUPLE algorithm is able to predict correctly coupling of light-activated control GPCRs and therefore to distinguish such receptor from bovine rhodopsin.

4.6.2.3 Human Class A oGPCRs

I run the PREDCOUPLE algorithm on all 64 human Class A oGPCRs both on their full sequences and their respective light-activated version

Gene name	Identifier	Full-length predicted coupling	Light-activated predicted coupling
GPR1	**MO3**	no match	Gα-i/o
GPR3	**MO4**	Gα-i/o	Gα-i/o
GPR4	**MO5**	Gα-i/o	Gα-i/o

GPR6	**MO6**	Gα-i/o	Gα-i/o
GPR12	**MO7**	Gα-i/o	Gα-i/o
GPR15	**MO8**	Gα-i/o	Gα-i/o
GPR17	**MO9**	Gα-i/o	Gα-i/o
GPR18	**MO10**	Gα-i/o	Gα-i/o
GPR19	**MO11**	Gα-i/o	Gα-i/o
GPR20	**MO12**	Gα-i/o	Gα-i/o
GPR21	**MO13**	Gα-s	Gα-i/o, Gα-q
GPR22	**MO14**	Gα-i/o	Gα-i/o
GPR25	**MO15**	Gα-i/o	Gα-i/o
GPR26	**MO16**	Gα-i/o	Gα-i/o
GPR27	**MO17**	Gα-i/o	Gα-i/o
GPR31	**MO18**	Gα-i/o	Gα-i/o
GPR32	**MO19**	Gα-i/o	Gα-i/o
GPR33	**MO20**	Gα-i/o	Gα-i/o
GPR34	**MO21**	Gα-i/o	Gα-i/o
GPR35	**MO22**	Gα-i/o	Gα-i/o, Gα-q
GPR37	**MO23**	Gα-i/o	Gα-i/o
GPR37L1	**MO24**	Gα-i/o	Gα-i/o
GPR39	**MO25**	Gα-i/o	Gα-i/o
GPR42	**MO26**	Gα-i/o	Gα-i/o
GPR45	**MO27**	Gα-i/o	Gα-i/o
GPR50	**MO28**	Gα-q, Gio, Gα-s	Gα-q
GPR52	**MO29**	Gα-i/o	Gα-i/o
GPR55	**MO30**	Gα-i/o	Gα-i/o
GPR61	**MO31**	Gα-i/o	Gα-i/o
GPR62	**MO32**	Gα-i/o	Gα-i/o
GPR63	**MO33**	Gα-i/o	Gα-i/o
GPR65	**MO34**	Gα-i/o	Gα-i/o
GPR68	**MO35**	Gα-i/o	Gα-i/o
GPR75	**MO36**	no match	Gα-i/o
GPR78	**MO37**	Gα-i/o	Gα-i/o
GPR82	**MO39**	Gα-i/o	Gα-i/o
GPR83	**MO40**	Gα-i/o, Gα-q	Gα-i/o
GPR84	**MO41**	Gα-i/o	Gα-i/o
GPR85	**MO42**	Gα-s	Gα-i/o
GPR87	**MO43**	Gα-i/o	Gα-i/o
GPR88	**MO44**	Gα-i/o	Gα-i/o
GPR101	**MO45**	Gα-i/o	Gα-i/o
GPR119	**MO46**	Gα-i/o	Gα-i/o
GPR120	**MO47**	Gα-i/o	Gα-i/o
GPR132	**MO4**	Gα-i/o	Gα-i/o
GPR135	**MO49**	Gα-q	Gα-i/o, Gα-q
GPR139	**MO50**	Gα-i/o	Gα-i/o
GPR141	**MO51**	Gα-q, Gα-i/o	Gα-i/o, Gα-q

GPR142	**MO52**	no match	Gα-i/o
GPR146	**MO53**	no match	Gα-i/o
GPR148	**MO54**	no match	Gα-i/o
GPR149	**MO55**	Gα-i/o	Gα-i/o
GPR150	**MO56**	Gα-i/o	Gα-i/o
GPR151	**MO57**	no match found	Gα-i/o
GPR152	**MO58**	Gα-i/o	Gα-i/o
GPR153	**MO59**	probable no gpcr	Gα-i/o
GPR160	**MO60**	Gα-i/o	Gα-i/o
GPR161	**MO61**	no match	Gα-i/o
GPR162	**MO62**	no match	Gα-i/o
GPR171	**MO63**	Gα-i/o	Gα-i/o
GPR173	**MO64**	Gα-i/o	Gα-i/o
GPR174	**MO65**	Gα-i/o	Gα-i/o
GPR176	**MO66**	Gα-i/o	Gα-i/o
GPR182	**MO67**	Gq	Gα-i/o
GPR183	**MO68**	no match	Gα-i/o

Table 4.16 Predicted G-Protein coupling for full-length control GPCRs according to the PREDCOUPLE algorithm

The PREDCOUPLE algorithm is not able to predict a possible G-Protein coupling for 8 out of 64 for full-length human Class A oGPCRs, while a possible coupling is always predicted for all 64 light-activated oGPCRs.

Out of 64 human Class A oGPCRs genes, the PREDCOUPLE algorithm show no agreement between the predicted coupling for full-length oGPCR and light-activated oGPCR genes in 14 cases.

The agreement between the full-length and light-activated oGPCRs genes predicted coupling can be explained by noticing that most of the genes are in both cases predicted to couple one single G-protein mediated pathways, Gα-i/o.

4.6.3 Validation of the PREDCOUPLE2 algorithm

4.6.3.1 Full-length control GPCRs

I run the PREDCOUPLE2 algorithm with the sequences of the full-length control GPCRs described in table 3.6 in order to estimate the algorithm performance with full-length GPCRs for which coupling properties are already known.

Control Receptor	Predominant G-coupling (Experimental)	Predicted G-Coupling
β2 adrenergic receptor (β2AR)	Gα-s	Gα-s
α1 adrenergic receptor (α1AR)	Gα-q	Gα-q
Free fatty acid receptor 3 (FFR3)	Gα-i/o	Gα-i/o
Adenosine A2A receptor (A2AR)	Gα-s	Gα-s
Dopamine receptor D1 (D1R)	Gα-s	Gα-s
Dopamine receptor D2 (D2R)	Gα-i/o	Gα-i/o
Muscarinic acetylcholine receptor M1 (M1R)	Gα-q and Gα-s	Gα-q
Muscarinic acetylcholine receptor M2 (M2R)	Gα-i/o	Gα-i/o
Muscarinic acetylcholine receptor M3 (M3R)	Gα-q	Gα-q

Table 4.17 Predicted G-Protein coupling for full-length control GPCRs according to the PREDCOUPLE2 algorithm

The PREDCOUPLE2 algorithm scored a 9/9 of correct predictions when analyzing sequences of full-length control GPCRs, showing that is able to predict correct coupling when analyzing entire sequences of GPCRs genes.

4.6.3.2 Light-activated control GPCRs

I run the PREDCOUPLE2 algorithm on the sequences of the light-activated control GPCRs to check if the algorithm is able to predict the correct coupling of chimeric GPCRs for which I have proved preservation of coupling properties in the experiment described in section 4.3.

Light-activated control Receptor	Predominant G-coupling (Experimental)	Predicted G-Coupling
β2 adrenergic receptor (β2AR)	Gα-s	Gα-s
α1 adrenergic receptor (α1AR)	Gα-q	Gα-q
Free fatty acid receptor 3 (FFR3)	Gα-i/o	Gα-i/o
Adenosine A2A receptor (A2AR)	Gα-s	Gα-s
Dopamine receptor D1 (D1R)	Gα-s	Gα-s
Dopamine receptor D2 (D2R)	Gα-i/o	Gα-i/o
Muscarinic acetylcholine receptor M1 (M1R)	Gα-q and Gα-s	Gα-q
Muscarinic acetylcholine receptor	Gα-i/o	Gα-i/o

M2 (M2R)		
Muscarinic acetylcholine receptor M3 (M3R)	Gα-q	Gα-q

Table 4.18 Predicted G-Protein coupling for full-length control GPCRs according to the PREDCOUPLE2 algorithm

The PREDCOUPLE2 algorithm scored a 9/9 of positive result when predicting G-Protein coupling for light-activated control GPCRs. All light-activated control GPCRS are predicted to be coupled equivalently to their full-length respective genes and in accordance to what I proved with the experiment described in section 4.3.

This result prove that the PREDCOUPLE2 algorithm is able to predict correctly coupling of light-activated control GPCRs and therefore to distinguish such receptor from bovine rhodopsin.

4.6.3.3 Human Class A oGPCRs

I run the PREDCOUPLE2 algorithm on all 64 human Class A oGPCRs both on their full sequences and in their respective light activated version.

Gene name	Identifier	Full-length predicted coupling	Light-activated predicted coupling
GPR1	**MO3**	Gα-q, Gα-i/o	Gio, Gα-q
GPR3	**MO4**	Gα-s, Gio	Gα-q, Gio
GPR4	**MO5**	Gio	Gio
GPR6	**MO6**	Gα-s	Gio, Gα-q, Gα-s
GPR12	**MO7**	Gα-s, Gio	Gα-q, Gio, Gα-s
GPR15	**MO8**	Gio, Gα-q, Gα-12/13	Gio, Gα-q, Gα-12/13
GPR17	**MO9**	Gio	Gio
GPR18	**MO10**	Gio	Gα-q, Gio
GPR19	**MO11**	Gα-q, Gio	Gα-q, Gio
GPR20	**MO12**	Gα-q, Gα-12/13, Gs	Gα-s, Gio
GPR21	**MO13**	Gio, Gα-s	Gio
GPR22	**MO14**	Gio, Gα-s, Gα-12/13	Gio
GPR25	**MO15**	Gio, Gα-12/13	Gio
GPR26	**MO16**	Gio, Gα-q	Gio, Gα-s
GPR27	**MO17**	Gio, Gα-q	Gio, Gα-q
GPR31	**MO18**	Gio	Gio

GPR32	**MO19**	Gio, Gα-q	Gio, Gα-q
GPR33	**MO20**	Gα-q	Gα-q, Gio
GPR34	**MO21**	Gio, Gα-q	Gio
GPR35	**MO22**	Gα-q, Gα-12/13, Gio	Gα-q, Gα-12/13, Gio
GPR37	**MO23**	Gα-q, Gαio, Gαs	Gα-q, Gαio
GPR37L1	**MO24**	Gio, Gα-s	Gio, Gα-q
GPR39	**MO25**	Gα-q	Gio, Gα-q
GPR42	**MO26**	Gio, Gα-q	Gio
GPR45	**MO27**	Gio	Gio, Gα-q
GPR50	**MO28**	Gio, Gα-s	Gio, Gα-s
GPR52	**MO29**	Gio, Gα-q	Gio
GPR55	**MO30**	Gio, Gα-q	Gio, Gα-q
GPR61	**MO31**	Gα-s,Gio	Gα-s, Gio
GPR62	**MO32**	Gα-s, Gα-q, Gα-12/13	Gio, Gα-s
GPR63	**MO33**	Gio, Gα-q	Gα-q, Gio
GPR65	**MO34**	Gio, Gα-q	Gα-q, Gio
GPR68	**MO35**	Gio	Gio
GPR75	**MO36**	Gio	Gio
GPR78	**MO37**	Gα-s, Gα-q	Gio, Gα-s
GPR82	**MO39**	Gα-q,Gio, Gα-12/13	Gα-q, Gio
GPR83	**MO40**	Gio, Gα-q	Gio, Gα-q
GPR84	**MO41**	Gio, Gα-s, Gα-12/13	Gio
GPR85	**MO42**	Gio, Gα-q	Gio
GPR87	**MO43**	Gio, Gα-q	Gio, Gα-q
GPR88	**MO44**	Gα-s, Gio	Gio, Gα-s
GPR119	**MO46**	Gio, Gα-q	Gio, Gα-q, Gα-12/13
GPR120	**MO47**	Gα-q, Gα-12/13, Gα-s, Gio	all - nothing
GPR132	**MO4**	Gio, Gq	Gα-q, Gio, Gα-s
GPR135	**MO49**	Gα-io, Gα-q	Gα-q, Gio
GPR139	**MO50**	no match	Gα-q, Gio
GPR141	**MO51**	Gα-q, Gio	Gio, Gα-q, Gα-12/13
GPR142	**MO52**	Gα-q, Gio, Gα-12/13, Gα-s	Gio
GPR146	**MO53**	Gio, Gα-q, Gα-12/13	Gα-q, Gio
GPR148	**MO54**	Gio	Gio
GPR149	**MO55**	Gio	Gio, Gα-q
GPR150	**MO56**	Gio, Gα-q, Gα-s	Gio
GPR151	**MO57**	Gio	Gio, Gα-s
GPR152	**MO58**	Gio	Gio
GPR153	**MO59**	probable no gpcr	Gio, Gα-q
GPR160	**MO60**	Gio, Gα-q	Gio
GPR161	**MO61**	Gio	Gio
GPR162	**MO62**	Gα-q, Gio	Gα-q, Gio
GPR171	**MO63**	Gα-q, Gio, Gα-12/13	Gio, Gα-q, Gα-12/13
GPR173	**MO64**	Gα-q, Gio, Gα-s	Gα-q, Gio
GPR174	**MO65**	Gio, Gα-q	Gio, Gα-q

GPR176	**MO66**	Gα-s	Gio, Gα-q, Gα-s
GPR182	**MO67**	Gα-q, Gio, Gα-12/13	Gα-q, Gio, Gα-12/13
GPR183	**MO68**	Gα-q, Gio	Gio, Gα-q

Table 4.19 Predicted G-Protein coupling for 64 human Class A human oGPCRs in their full-length and light-activated form according to the PREDCOUPLE2 algorithm

The PREDCOUPLE2 algorithm is able to predict a possible G-Protein coupling for all 64 full-length and light-activated orphan GPCRs.

Out of 64 human Class A oGPCRs genes, the PREDCOUPLE algorithm show no agreement between the predicted coupling for full-length oGPCR and light-activated oGPCR genes in 11 cases.

The agreement between the full-length and light-activated oGPCRs genes predicted coupling is helped by the ability of the PREDCOUPLE2 algorithm to propose more than one single possible pathways.

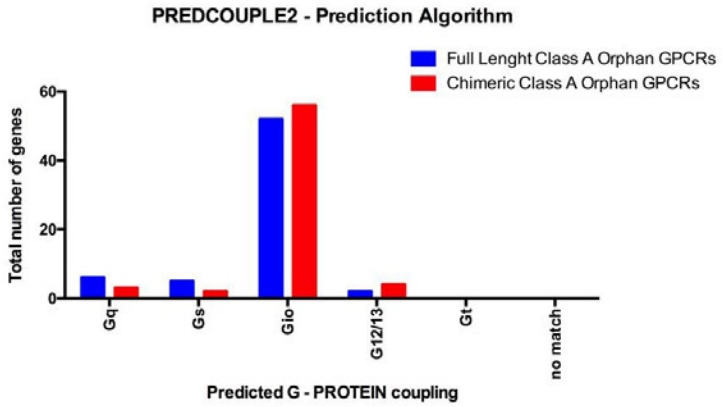

Figure 4.27 Predicted G-Protein coupling for 64 full-length and relative light-activated human Class A oGPCRs according to the Predcouple2 Algorithm

4.6.4 Comparison between the different a l g o r i t h m s

I defined for each control and oGPCRs in both full-length and light-activated chimeric form the agreement between the predicted couplings between different algorithms as the superposition between the predicted couplings for the two most probable pathways.

In the case of full-length control GPCRs, there is full agreement both considering only the most probable pathways and the two most probable

pathways considering PREDCOUPLE vs GRIFFIN and PREDCOUPLE2 vs GRIFFIN.

In the case of the light-activated control GPCRs, there is no agreement between the PREDCOUPLE, PREDCOUPLE 2 and GRIFFIN, and this due to the fact that for the GRIFFIN algorithm all light-activated chimeric receptors (control and oGPCRs) are considered to conserve the coupling properties of rhodopsin.

I did not perform the agreement analysis between the PREDCOUPLE and the PREDCOUPLE 2 being two different version of the same algorithm, and thus a strong agreement between the predicted couplings of these two algorithm would be less significant.

In Figure 4.27 and 4.28 the number of positive coupling (superposition between the different algorithms) considering only the main predicted pathways and negative coupling (no superposition between the different algorithms) are shown for PREDCOUPLE vs GRIFFIN and PREDCOUPLE2 vs. GRIFFIN for the full-length 64 human Class A oGPCRs.

In the case of PREDCOUPLE vs GRIFFIN there is high disagreement between the predicted couplings for full-length oGPCRs both considering only the main predicted pathways or the two most probable. This disagreement is present also when comparing PREDCOUPLE2 vs GRIFFIN, although in the case of the PREDCOUPLE vs GRIFFIN, I got more positive than negative coupling.

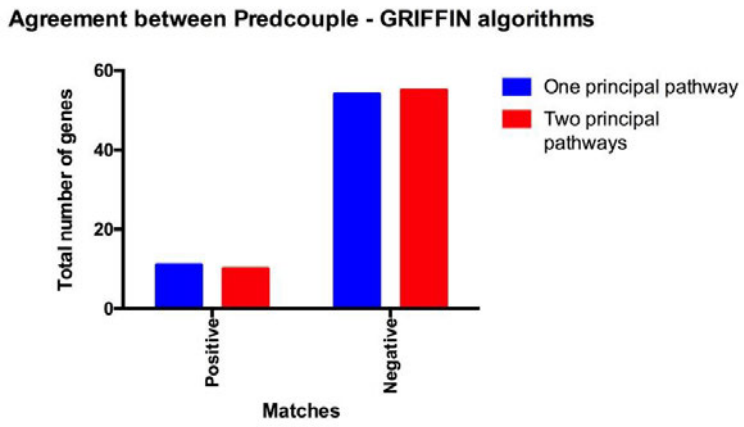

Figure 4.28 Number of positive and negative matches between the Predcouple and Griffin Algorithms for principal and first two principal predicted G-Protein coupling for 64 full-length and relative light-activated human Class A oGPCRs

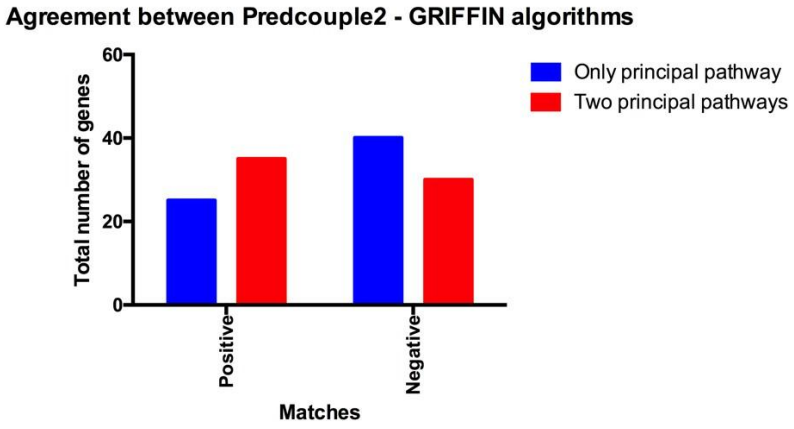

Fig 4.29 Number of positive and negative matches between the Predcouple and Griffin Algorithms for principal and first two principal predicted G-Protein coupling for 65 full-length and relative light-activated chimeric Class A oGPCRs

5 - Discussion

Orphan GPCRs (oGPCRs) represent a valuable target in drug discovery due to their proven and potential role in many diseases. Till now, exploiting of oGPCRs in drug development was limited by the insufficient amount of information about these receptors, including limited knowledge about their signaling and structural properties.

Notably, in some cases non signaling functions or ligand independent functions have been proposed for oGPCRs, but it still remains a question about if and what signaling cascades these receptors activate once stimulated by an agonist.

I have shown that the chimeric approach is a new approach to increase the amount of information available about the functional and signaling properties of orphan GPCRs and guide drug discovery into exploring new possible therapeutic approaches.

Although the design of chimeric GPCRs has been largely focused in the last 15 years on the study their molecular functions, this approach has been limited by the lack of a systematic protocol in order to design, clone and test large libraries of chimeric receptors. Also, the limited amount of data about the functionality of chimeric GPCRs has brought doubts about the validity of the domain swapping dogma and more generally on the idea that GPCRs can be considered as receptors in which the ligand interaction and the signal transduction are two related independent processes. Also, for the large number of chimeric GPCRs engineered in the past, a platform for a complete screening of all G-Protein mediated signaling pathways was missing, leaving the concern that functional chimeric GPCRs might show original unwanted coupling properties in comparison with their full-length receptors. In this work I demonstrated that such a systematic approach to the design, clone and screen large libraries of chimeric receptors is possible.

Indeed, the “chimeric algorithm” described in this thesis allows creating libraries of chimeric GPCRs in a step-by-step process from sequence retrieval till the generation of the synthetic genes.

The gene reporter system based on the use of luciferase-based reporter plasmids allowed screening for several G-Protein mediated pathways for a large number of chimeric receptor at the same time. For all G-Protein mediated pathways different methods are currently available. However, they have the inconvenient of being based on different readouts. This implies that, while screening for activation of different G-Protein pathways, results coming from different assays cannot be easily compared.

The testing platform build on the use of luciferase based gene reporter plasmids has the unique advantage of unifying in one single output signals coming from different signaling pathways. In fact, one of the main problems when screening large libraries of receptors that can activate different signaling pathways, such as GPCRs, is comparing results coming from different screening systems.

The reliability of the luciferase-based assays is testified by the results on ligand-activated control GPCRs, which show activation pattern in accordance with previously available data, implying that the platform is able to correctly detect and distinguish G-Protein mediated signaling pathways.

The reliability of the “chimeric algorithm” is testified by the results achieved with the testing of the light-activated control GPCRs library.

Light-activated version of full-length control GPCRs designed following the “chimeric algorithm” show intact functionality and specificity, excluding most of the concern regarding the generation of chimeric GPCRs such as loss of functionality or creation/deletion of signaling cascades.

The cloning approach based on the design of a common mother construct for the creation of all genes of a synthetic library can be translated for application with any other GPCRs, such as GPCRs or light sensitive opsin that might present more convenient properties. Bi stable opsins as well as protein that present a close life cycle can be used following the same approach to create libraries of light-activated receptors tuned to fit to different experimental

settings. One of the biggest concerns at the beginning of this work was to develop a screening platform that allows me to overcome the issue of not having a proper positive control while testing the light-activated human Class A oGPCRs. The coherence between the screening data belonging to full-length control GPCRs and their relative light-activated chimeric versions allow me to propose that the signaling data collected from the light-activated human Class A oGPCRs reflect the signaling properties of their relative full-length genes.

Out of the 64 light-activated human Class A oGPCRs tested for G-Protein mediated pathways, I observed in total 19 receptor-pathway couplings in the HEK293 cell line. Considering that G-Protein coupling properties can be cell- type dependent, these results strongly suggested that human Class A oGPCRs might still conserve intact functional properties and are able to signal trough G- Protein mediated pathways even if I was not able to detect a significant activation in HEK293 cells. Fourteen different light-activated human Class A oGPCRs designed according the chimeric algorithm show significant activation of one or more luciferase-based reporter plasmids.

Light-activated human Class A oGPCR	Luciferase-based reporter plasmid activated	Possible second messengers activated
Opto-**GPR1**	CRE	cAMP é
Opto-**GPR3**	SRE	PKC and MAPK/ERK
Opto-**GPR4**	CRE + NECA	cAMP ê
Opto-**GPR18**	SRE	PKC and MAPK/ERK
Opto-**GPR21**	CRE	cAMP é
Opto-**GPR32**	CRE	cAMP é
Opto-**GPR33**	SRE.L, SRE, CRE + NECA	RhoA, PKC and MAPK/ERK, cAMP ê
Opto-**GPR42**	CRE	cAMP é
Opto-**GPR55**	CRE + NECA	cAMP ê
Opto-**GPR63**	CRE + NECA	cAMP ê
Opto-**GPR68**	SRE	PKC and MAPK/ERK
Opto-**GPR78**	SRE	PKC and MAPK/ERK
Opto-**GPR88**	SRE	PKC and MAPK/ERK
Opto-**GPR135**	CRE	cAMP é

Table 5.1 List of light-activated human Class A oGPCRs with significant activation of one or more luciferase-based reporter plasmid upon light stimulation.

The functional data I collected can be compared to the already available information for the receptors listed in table 4.1. Constitute activity and intensity and localization of oGPCR expression are important factors in order to estimate oGPCR possible physiological roles. Information coming from knockout and knockdown studies are as well fundamental.

Especially taking in account the importance of GPCRs in drug discovery, the availability of murine knockout models is extremely important in understanding the physiological functional roles of GPCRs and oGPCRs and to observe disease-related phenotypes.

In the following table are collected the available KO murine models (in the form of Embryonic Steam (ES) cells or stable murine strains) with relative phenotype (when observed). In Appendix II, references reporting phenotypes are collected (from the International Mouse Phenotyping Consortium (IMPC) and the Jackson Laboratories database).

	Gene	**ES**	**Mice**	**Phenotype**	**Phenotype description**
1	GPR1	YES	YES	YES	Metabolic-glutamate intolerance
2	GPR2 (Ccr10)	YES	Under Production	YES	Behavior, Immune response, Growth
3	GPR3	YES	Under Production	YES	Behavior, Metabolism
4	GPR4	YES	Under Production	Proposed	Cardiovascular system, Homeostasis, Aging, Respiration
5	GPR6	YES	Under Production	Proposed	Behavior, Nervous system
6	GPR7 (Npbwr1)	NO	YES	Proposed	Behavior, Growth, Liver and Biliary system
7	GPR14 (Uts2r)	YES	YES	Proposed	Cardiovascular system, Muscular system
8	GPR17	YES	YES	Proposed	Behavior, Immune system, Aging, Nervous system
9	GPR18	YES	Under Production	NO	NO
10	GPR19	YES	YES	Proposed	Behavior
11	GPR20	YES	YES	YES	Behavior, Nervous system, Immune system, Aging
12	GPR21	YES	Under Production	Proposed	Behavior, Growth, Metabolism, Immune system
13	GPR22	YES	YES	YES	Cardiovascular system, Metabolism
14	GPR23 (Lpar4)	YES	YES	Proposed	Embryogenesis, Growth, Immune system, Aging, Cardiovascular system
15	GPR24 (Mchr1)	YES	NO	Proposed	Behavior, cardiovascular system, growth, nervous system
16	GPR26	YES	Under Production	Proposed	Behavior, Metabolism, Growth
17	GPR27	NO	Under Production	NO	NO
18	GPR30	YES	Under Production	YES	Cardiovascular system, Growth, Immune system, Biliary system

19	GPR31b	YES	Under Production	NO	NO
20	GPR33	YES	YES	YES	Nervous system (abnormal gait)
21	GPR34	YES	YES	YES	Immune system, Nervous system, Vision
22	GPR35	YES	YES	NO	NO
23	GPR37	YES	Under Production	Proposed	Behavior, Growth, Nervous system
24	GPR37l1	YES	Under Production	Proposed	Behavior, Nervous system, Vision
25	GPR39	NO	YES	Proposed	Digestion, Growth, Metabolism
26	GPR40 (Ffar1)	YES	YES	YES	Behavior, Metabolism, Biliary system, Nervous system, Taste
27	GPR41 (Ffar3)	YES	NO	Proposed	Cardiovascular system, Growth, Metabolism, Nervous system
28	GPR43 (Ffar2)	YES	YES	Proposed	Digestive system, Growth, Metabolism, Immune system, Aging
29	GPR44 (Ptgdr2)	YES	NO	Proposed	Hematopoietic system, Metabolism, Immune system
30	GPR45	YES	NO	NO	NO
31	GPR48 (Lgr4)	YES	NO	Proposed	Digestive system, Embryogenesis, Growth, Metabolism, Immune system, Aging, Nervous system, Vision
32	GPR49 (Lgr5)	YES	Under Production	Proposed	Behavior, Digestive system, Growth, Metabolism, Aging,
33	GPR50	YES	YES	YES	Behavior, Growth, Metabolism
34	GPR51 (Gabbr2)	NO	YES	Proposed	Behavior, Growth, Metabolism, Aging, nervous system
35	GPR54 (Kiss1r)	YES	YES	YES	Behavior, Growth, Digestive system, Metabolism, Immune system, Aging, Nervous system
36	GPR55	YES	YES	YES	Metabolism, Immune system, Skeleton
37	GPR56 (Adgrg1)	NO	Under Production	Not confirmed	Behavior, Nervous system
38	GPR58 (Taar2)	YES	YES	NO	NO
39	GPR61	NO	YES	Proposed	Metabolism (aldosterone)
40	GPR62	YES	Under Production	NO	NO
41	GPR63	YES	YES	NO	NO
42	GPR64 (Adgrg2)	YES	Under Production	Proposed	Metabolism, Reproduction
43	GPR65	YES	YES	YES	Immune system, Hematopoietic system, Skeleton
44	GPR66 (Nmur1)	YES	YES	YES	Metabolism
45	GPR68	NO	Under Production	Proposed	Hematopoietic system, Metabolism, Immune system, Tumorigenesis
46	GPR69 (Lancl1)	YES	Under Production	Proposed	Metabolism, Immune system, Nervous system
47	GPR70 (Tas1r1)	YES	Under Production	Proposed	Nervous system, Reproduction, Taste/Olfaction
48	GPR71 (Tas1r2)	YES	Under Production	Proposed	Nervous system, Taste/Olfaction
49	GPR73 (Prokr1)	YES	YES	YES	Behavior, Cardiovascular system, Metabolism
50	GPR73l1 (Prokr2)	YES	YES	Proposed	Behavior, Growth, Metabolism, Aging, Nervous system,

51	GPR74	NO	Under Production	Proposed	Nervous system
52	GPR75	YES	Under Production	NO	NO
53	GPR77 (C5ar2)	YES	Under Production	Proposed	Behavior, Metabolism, Biliary system, Aging
54	GPR80 (Oxgr1)	YES	YES	Proposed	Metabolism, Immune system, Renal system
55	GPR81 (Hcar1)	YES	NO	Proposed	Metabolism
56	GPR83	YES	Under Production	Proposed	Behavior, Hematopoietic system, Immune system
57	GPR84	YES	YES	Proposed	Hematopoietic system, Immune system
58	GPR85	YES	Under Production	Proposed	Behavior, Growth, Nervous system
59	GPR86 (P2ry13)	YES	YES	Proposed	Digestive system, Metabolism, Biliary system, Skeleton
60	GPR87	YES	YES	Proposed	Limbs, Digits, Tail, Skeleton
61	GPR88	YES	YES	Proposed	Behavior, Nervous system
62	GPR89	YES	Under Production	Proposed	Growth, Metabolism, Immune system, Aging, Pigmentation, Reproduction
63	GPR90 (Mrgprh)	YES	Under Production	NO	NO
64	GPR91 (Sucnr1)	YES	Under Production	Proposed	Hematopoietic system, Immune system, Renal system
65	GPR93 (Lpar5)	YES	YES	YES	Behavior, Nervous system
66	GPR97	YES	NO	NO	NO
67	GPR98 (Adgrv1)	YES	YES	YES	Behavior, Hearing system, Aging, Nervous system, Vision
68	GPR100 (Rxfp4)	YES	YES	YES	Behavior, Growth
69	GPR101	YES	YES	NO	NO
70	GPR103 (Qrfpr)	YES	YES	Proposed	Skeleton
71	GPR106 (Rxfp2)	YES	YES	YES	Reproduction, Skeleton
72	GPR107	YES	YES	YES	Hematopoietic system, Metabolism, Aging
73	GPR108	YES	YES	NO	NO
74	GPR110 (Adgrf1)	YES	Under Production	NO	NO
75	GPR111 (Adgrf2)	YES	NO	NO	NO
76	GPR112 (Adgrg4)	YES	Under Production	NO	NO
77	GPR114 (Adgrg5)	YES	YES	NO	NO
78	GPR115 (Adgrf4)	YES	YES	NO	NO
79	GPR116 (Adgrf5)	YES	YES	YES	Growth, Hematopoietic system, Metabolism, Immune system, Aging, Respiratory system
80	GPR119	YES	YES	NO	NO
81	GPR120 (Ffar4)	YES	YES	Proposed	Growth, Metabolism, Biliary system, Nervous system, Taste/Olfaction
82	GPR123 (Adgra1)	YES	YES	NO	NO

83	GPR124	NO	YES	Proposed	Cardiovascular system, Digestive system, Growth, Aging, Nervous system, Respiratory system
84	GPR125 (Adgra2)	YES	Under Production	NO	NO
85	GPR126 (Adgrg6)	YES	NO	Proposed	Behavior, Growth, Metabolism, Aging, Nervous system
86	GPR127	YES	YES	NO	NO
87	GPR128 (Adgrg7)	YES	YES	NO	NO
88	GPR131 (Gpbar1)	YES	YES	YES	Behavior, Growth, Metabolism, Biliary system
89	GPR132	YES	Under Production	Proposed	Growth, Hematopoietic system, Immune system, Respiratory System
90	GPR133	YES	YES	NO	NO
91	GPR136 (Opn5)	NO	Under Production	NO	NO
92	GPR137	YES	Under Production	NO	NO
93	GPR137b	YES	YES	NO	NO
94	GPR137c	YES	NO	NO	NO
95	GPR139	YES	YES	NO	NO
96	GPR142	YES	YES	NO	NO
97	GPR143	YES	NO	Proposed	Nervous system, Pigmentation, Vision
98	GPR146	YES	NO	NO	NO
99	GPR149	YES	Under Production	Proposed	Behavior, Reproduction
100	GPR150	YES	NO	NO	NO
101	GPR152	YES	YES	NO	NO
102	GPR153	YES	Under Production	NO	NO
103	GPR154 (Npsr1)	YES	YES	Proposed	Metabolism, Respiration
104	GPR155	YES	NO	NO	NO
105	GPR156	YES	YES	NO	NO
106	GPR157	YES	NO	NO	NO
107	GPR158	YES	YES	NO	NO
108	GPR160	YES	NO	NO	NO
109	GPR161	YES	NO	Proposed	Behavior, Embryogenesis, Aging, Nervous system, Vision
110	GPR162	YES	YES	NO	NO
111	GPR165	NO	Under Production	NO	NO
112	GPR171	YES	NO	NO	NO
113	GPR172b (Slc52a2)	YES	YES	NO	NO
114	GPR175)	YES	YES	NO	NO
115	GPR176	YES	YES	NO	NO
116	GPR178	YES	YES	NO	NO
117	GRP179	YES	Under Production	Proposed	Vision
118	GPR180	YES	Under Production	Proposed	Cardiovascular system, Metabolism
119	GPR182	YES	YES	NO	NO

120	GPR183	YES	YES	YES	Hematopoietic and Immune system
121	GRM1	NO	Under Production	YES	Behavior, Growth, Hearing, metabolism, Immune system, Aging, Nervous system
122	GRM2	YES	Under Production	YES	Behavior, Metabolism, Nervous system
123	GRM3	YES	YES	YES	Behavior, Nervous system
124	GRM4	NO	Under Production	Proposed	Behavior, Nervous system
125	GRM5	NO	Under Production	YES	Behavior, Growth, Nervous system
126	GRM6	YES	YES	YES	Nervous system, Vision
127	GRM7	YES	NO	YES	Behavior, Metabolism, Aging, Nervous system
128	GRM8	YES	YES	YES	Behavior, Growth, Metabolism, Nervous system
129	GPRC2a	YES	YES	YES	Behavior, Growth, Metabolism, Immune system, Aging Vision
130	GPRC5a	YES	NO	YES	Immune system, Respiration, Tumorigenesis
131	GPRC5b	YES	YES	YES	Behavior, Aging

Table 5.2 List of GPCRs (including oGPCRs) belonging to different subfamilies (Class A, Adhesion and Class C) for which at least one KO model is available or under development.

The data in table 4.2 show that for the moment a consistent amount of murine KO models are currently available for GPCRs. This might change in the future thanks to the CRISP/CAS9 technique that shortens both costs and time of development of stable KO strains[261,262]. For the oGPCRs for which I detected a significant stimulation of one or more luciferase-based reporter plasmids the previously mentioned information is also available. For some specific cases possible agonist and related activated second messengers are proposed in single studies, explaining why these genes are still formally considered as oGPCRs (see table 5.3 for genes and references).

In the following paragraphs, for each oGPCRs for which I detected at least one possible G-Protein coupling, I will summarize what is known and what my result add to it.

GPR1 is currently considered a chemerin receptor, although an agreement on this is still missing. Chemerin is a chemoattractant protein that plays an active role in recruitment of dendritic cells and macrophages to inflammation sites. A chemerin-dependent activation of RhoA has been reported for GPR1, not recorded in my assays. However, the light-activated GPR1 showed significant activation of the CRE luciferase-based plasmid, suggesting that GPR1

increases intracellular levels of cAMP. Modifications of intracellular level of cAMP are required for regulation of actin based cell migration and chemotaxis. Thus activation of the CRE luciferase-based reporter plasmid is in agreement with the hypothesis of the involvement of this receptor in inflammation and migration-related biological processes. GPR3 is expressed in oocytes and it is supposed to play a role in the communication between oocytes and the surrounding stromal tissue. It has been proved its ability to increase cAMP in oocytes without the interaction with a ligand (constitutive activity), although Sphingosine 1-phosphate (S1P) has been proposed as putative ligand. Recently, it has been suggested that GPR3 might play a role in the Alzheimer's disease[263]. It has been determined that the absence of GPR3 alleviated the cognitive deficits and reduced amyloid pathology in four different d i s e a s e s -
relevant mouse models of Alzheimer. Furthermore, GPR3 was found to be elevated in postmortem brain tissue from a subset of patient suffering of Alzheimer[264]. The activation of the CRE luciferase-based reporter system by the light-activated GPR3 supports the hypothesis that this receptor might modify intracellular level of cAMP, not only in oocytes. For this receptor, as well as for all other oGPCRs involved in diseases, the conservation of the activated pathway in literature and in my screening assay open to different considerations. The look for a possible agonist or antagonist for GPR3 could be direct to ensemble of molecules that have been proved to act as ligand for similar GPCR that modify as well intracellular level of cAMP. The screening could be limited even more by considering only molecules present in the native tissue of GPR3 or similar GPCRs, conserving the approach of the already described orphan strategy. On the other hand, both the information coming from my screening assay and confirm by literature might move the therapeutic approach from GPR3 through the pathway activated by the receptor itself. My results suggest that the role of cAMP regulation in Alzheimer disease should be further investigate and that this second messenger could be considered at therapeutic target.

GPR4 is considered to be a proton-sensing GPCR. This class of GPCRs is supposed to be activated usually upon pH drops to values between 6.2-6.8. The study of proton-sensing GPCRs is still challenging (although different knockout murine models are available, see table 5.2) for the redundancy of this class of receptors that usually call for multiple gene deletions. Previous studies proved the ability of GPR4 to activate different G-protein mediated pathways, while in my screening assay the light-activated GPR4 is proved to lower significantly intracellular level of cAMP. pH modification is involved in inflammatory processes. The light-activated GPR4 would allow investigating further the role of this specific proton-sensing GPCR in tissue where different other proton- sensing GPCRs are simultaneously expressed. It is interesting to notice that this receptor, being involved in pH sensing and (directly to indirectly) to inflammatory states shown ability to modify intracellular level of cAMP in my screening assay, raising considerations similar to the ones related to GPR3. GPR18 is supposed to be able increase intracellular level of cAMP upon activation with lipoamino acids and cannabinoids. The hypothesis that GPR18 upon stimulation with different ligands might be able to modify cAMP intracellular levels fit with the available data about its possible physiological
roles. GPR18 is considered involved in cell migration[280], macrophage apoptosis [281] and it has been proposed a role in controlling the reconstitution of the mouse small intestine intraepithelial lymphocytes [282].

I found activation of the SRE reporter by the Opto-GPR18, while no signal was detected in experiments with the CRE luciferase-based reporter plasmid. Indeed, activation of the ERK1/2 pathway by GPR18 has been reported in the control of hypotension in rats. Also, the MAPK/ERK1/2 pathway is involved in cell proliferation and cell death, and as previously described GPR18 might be linked to macrophage apoptosis.

Gene	Tissue Localization	KO (ES or mice)	Phenotype	Possible agonists	Second messengers
GPR1	Ubiquitous	YES	YES	Chemerin	RhoA, ROCK[265]
GPR3	Ubiquitous	YES	Not confirmed	S1-P	cAMP[266]
GPR4	Ubiquitous	YES	Not confirmed	Protons	cAMP, RhoA, PKC[267, 268,269]
GPR18	Ubiquitous	YES	Not confirmed	Lipoamino acids	cAMP[270, 271]
GPR21	Ubiquitous	YES	Not confirmed	No information available	PKC[272]
GPR32	Arterial/venous tissue	No		No information available	No information available
GPR33	Thyroid, lung, spleen, thymus	YES	YES	No information available	cAMP[273]
GPR42	No signal in human tissue	No		Propionate	cAMP[274]
GPR55	Brain and digestive system	YES	YES	Cannabinoid ligands	PKC and MAPK/ERK1[275]
GPR61	CNS and testes	YES	Not confirmed	No information available	cAMP[155]
GPR63	Ubiquitous	YES	No	Sphingosine 1-phosphate	No Information available
GPR68	Ubiquitous	YES	YES	Sphingosylphosphorylcholin, Benzodiazepine, Protons	cAMP, PKC [276,277, 278]
GPR78	Brain and placenta	NO		Constitute activity	cAMP[279]
GPR88	Central Nervous System and trachea	YES	YES	No information available	No information available
GPR135	Ubiquitous, but especially CNS	No		No Information available	No information available
GPR150	Mainly CNS	YES	Not confirmed	No Information available	No information available

Table 5.3 List of human class A oGPCRs that show significant activation of one or more luciferase-based reporter plasmid including information about tissue expression, presence of a murine knockout with significant phenotype, list of possible candidate agonists and G-protein mediated pathway for which coupling was demonstrated using different assays.

I found a significant number of light-activated human Class A oGPCRs able to activate directly or inversely the CRE reporter plasmid, implying their ability to modify intracellular cAMP levels, fundamental in regulating actin based cell migration, chemotaxis and in general important for the control of inflammatory processes.

GPR32 and GPR33 belong to this group of light-activated oGPCRs that activate the CRE reporter plasmid.

GPR32 has been proposed to be sensitive to resolvins, a family of lipid mediators involved in temper the inflammatory response. More specifically, GPR32 seems to be involved in inflammatory processes localized in the lungs, such as inflammatory signaling in human airway epithelial cells and in the TGF- β1 induced epithelial mesenchymal transition of lung cancer cells.

GPR33, although still classified as an oGPCRs, is considered by sequence homology as a chemoattractant receptor; its inactivation in humans, different great apes and rodent species as well as the presence of an intact allele in geographically restricted human populations suggest that this gene was undergoing positive selection in the last million years[273].

Our data proves signaling functions for the intact light-activated GPR33 both in cAMP and internalization related G-Protein mediated pathways, strengthening the hypothesis of an active role of GPR33 in leucocyte chemotaxis and pathogen entry, like similar chemoattractant receptors, although further internalization studies might be needed in order to verify this hypothesis.

GPR42 can be considered the oGPCRs included in my screening with the most limited amount of information available. Expression data for this oGPCR are not available as well as KO models. It has been suggested its ability to increase levels of intracellular cAMP after stimulation with Propionate, ability recorded also in my data for the Opto-GPR42[283].

Unlike GPR42, GPR55 is an oGPCR for which the available information justifies its involvement in several different physiological roles. It has been shown for GPR55 the ability to activate PKC and MAPK mediated pathways. These pathways are involved in cell proliferation: indeed, for GPR55 it has been

proposed a role in neuronal growth as well as in angiogenesis. In my screening Opto-GPR55 reversely activates the CRE luciferase-based reporter plasmid, the ability of GPR55 to modify intracellular level of cAMP might strengthen the hypothesis that this oGPCRs is involved in the inflammatory response and migration-related molecular processes. In fact, it has been shown that GPR55 is involved in intestinal inflammation, in lipid accumulation and consequent rise of inflammatory states and in the process of cancer angiogenesis in ovarian carcinoma. Actually, the role of GPR55 is not limited to the angiogenic process, but it has been shown that GPR55 promotes cancel cell proliferation, metastasis formation in colon cancer and in general GPR55 is considered a novel tumorigenic target[284]. All previous considerations suggest that GPR55 might be able to activate different G-protein mediated pathways and play a relevant role in different physiological processes.

GPR61 is known to be constitutive active and to have the ability to increase intracellular levels of cAMP. Opto-GPR61 significantly activate the CRE luciferase-reporter plasmid, confirming the coupling properties of this oGPCR. From a functional point of view, little is known about GPR61; the current hypothesis suggests a role of GPR61 in metabolism and in the regulation of aldosterone secretion.

Although for GPR63 candidate agonists were identified[263], limited amount of information is available on its involvement in any relevant physiological processes. Opto-GPR63 was able to significantly reduce the amount of intracellular cAMP, suggesting that this oGPCR might possible be involved in inflammatory processes or, in general, in biological processes involving cell migration.

For GPR68 new information about possible agonist was recently retrieved using approaches based on homology models, leading to the identification of benzodiazepine as novel not-selective agonist. GPR68 is considered to be a proton-sensing GPCR (like the previously described GPR4) involved in different physiological processes related to acidosis, such as inflammation and the regulatory processes of the acidic tumor microenvironment. Dendritic cells

expressing GPR68 show inhibited migration properties in acidic environments. GPR68 controls the activity of proton transport proteins in epithelial cells and plays a specific role in asthma, regulating the contraction of airways in respect to change in pH[285]. For GPR68 example of cAMP and PKC mediated pathway activation are present in literature. In my screening the Opto-GPR68 activated the SRE luciferase-based reporter plasmid, while no significant activation was measured for the CRE luciferase-based reporter. The data of my screening compared with previously known data about GPR68 suggest that the ability of GPR68 to activate different G-protein mediated pathway might be related not only on the ligand and tissue dependency, but also on the difference between homeostatic and inflammatory conditions.

GPR78 presents a unique feature among the oGPCRs for which I recorded a significant light-dependent activation in my screening. In fact, it is the only gene expressed in humans, but not in mice. GPR78 has been suggested to be constitutive active in HEK293 cells, increasing the amount of intracellular cAMP levels. In my screening actually two different reporter plasmid were significantly activated by the Opto-GPR78, the SRE and the SRE.L luciferase-based reporter plasmid, while no significant activation was measured for the luciferase-based CRE reporter plasmid. This suggest that possibly the increase of cAMP level related to constitutive activity of GPR78 might not be related to this receptor function.

Most of the oGPCRs positive in my screening were found to have either inflammation-cAMP related signaling functions or cell growth-apoptosis related functions. Actually, oGPCRs were believed to be involved in high-order brain functions both taking in account the difficulties in finding both proper candidate agonists and, as shown in table 4.2, clear phenotypes in mouse KO studies. In the case of GPR88, the hypothesis of its implication in high-order brain function, more specifically disease models, is still valid. Mice lacking GPR88 exhibit a lower basal level of dopamine, which is a fundamental regulator of brain activity[137,138]. GPR88 is supposed to be involved in disorders of the CNS such as the bipolar disorder (BD) and schizophrenia[139]. Relation between GPR88

and these mental illnesses were found in different human populations where the relation was analyzed in triads formed by parents and child. In my screening, opto-GPR88 activates the SRE luciferase-based reporter plasmid, indicating the ability of GPR88 to activate either PKC or MAPK mediated pathways. However, how the activation of these specific pathways might lead to the development of sever physiological disease is difficult to estimate.

GPR135 and GPR150 can be considered a similar case to GPR42. Also for this oGPCR very little is known apart from its expression pattern in human. No possible candidate agonist is available, as well as hypothesis about their physiological role. Opto-GPR135 activates the CRE reporter plasmid, implying the ability of GPR135 to increase intracellular level of cAMP. The Opto-GPR150 decreased the CRE luciferase-based signal of NECA pre-stimulated cells, implying the ability of GPR150 to decrease intracellular level of cAMP. The absence of KO models as well as putative agonists makes the light-activated version of these oGPCRs a promising approach to dissect their physiological roles in the native tissues.

What implications does this research result have? For the light-activated human Class A oGPCRs for which I did not detect functional coupling a conserved functionality cannot be excluded. Being the G-Protein coupling dependent on the intracellular concentration of G-Protein related proteins, those light-activated human oGPCRs for which no significant coupling was detected in HEK293 cells could still activate canonical and non-canonical pathways in other cell types and tissues.

This data inspire further experiments in which light-activated Class A human oGPCRs can be tested in native tissue/organs, allowing to dissect their physiological roles. In fact, the light-activated oGPCRs may for the first time allow to test in vitro and in vivo the functional properties of this class of GPCRs using all advantages the Optogenetic approaches.

The data I collected allow to prioritize the study of oGPCRs in specific disease models by knowing their signaling properties before elucidating their activation mechanism and identifying proper agonists.

More generally, the approach described in this work permits to further elucidate the role of G-Protein dependent specific signaling pathways and second messenger. The creation of light-activated versions of known GPCRs will allow establishing a technique in which these pathways can be turned ON and OFF. This might allow in further studies to relate specific phenotypes to signaling pathways.

Finally, the rhodopsin-based library of chimeric GPCRs I designed may allow investigating structure properties of GPCRs for which current structure knowledge is limited; in fact, the techniques and the results achieved in crystallization of rhodopsin in its active and inactive form, as well as a coupled taste with the G-Protein can be as well used to obtain structural information about orphan GPCRs and verified their coupling properties by crystallization studies.

In summary, the chimeric algorithm as well as the luciferase-based platform represent a new approach for elucidating functional properties for oGPCRs for which little is knew besides sequences and expression pattern.

The alternative approach based on the study of gene sequences using bioinformatics tools such as G-Protein prediction algorithms based on different mathematical and statistical tools in order to predict G-Protein coupling is far from being able to substitute functional testing. In fact, the algorithms tested in this work, when they do not fail in distinguish full-length GPCRs from their respective light-activated chimeric receptors, give not coherent results, showing a prediction pattern dependent on the mathematical or statistical approaches used to calculate the prediction. Also, these algorithms do not take in account differences in the topological localization of specific sequences when predicting G-Protein coupling, and approximate properties with a degree of detail not sufficient for elaborate proper predictions.

The main aim of this work was to answer three questions:

- ***Are orphan GPCRs functional at all?***
- ***If yes, which signaling pathways do they activate?***

- ***It is possible to retrieve a possible ligand for the receptors still consider as orphan?***

My results show that some Class A oGPCRs are functional and able to activate G-Protein mediated pathways in light-activated chimeras. The stability and reliability of both the chimeric algorithm and the assay strongly suggest that the signaling properties recorded for the light-activated human Class A oGPCRs reflect the signaling properties of their respective full-length genes. My results suggest that further investigation is needed for those light-activated human Class A oGPCRs for which no significant G-Protein coupling was recorded, with necessary testing in native tissues. The chimeric approach and my library of light-activated Class A human oGPCRs allow to overcome the necessity for information about activation properties and agonist for oGPCRs by creating a class of signaling-equivalent synthetic receptor that can be used in vitro and in vivo to elucidate signaling properties and physiological roles of this fundamental class of receptors.

6 - Appendix I

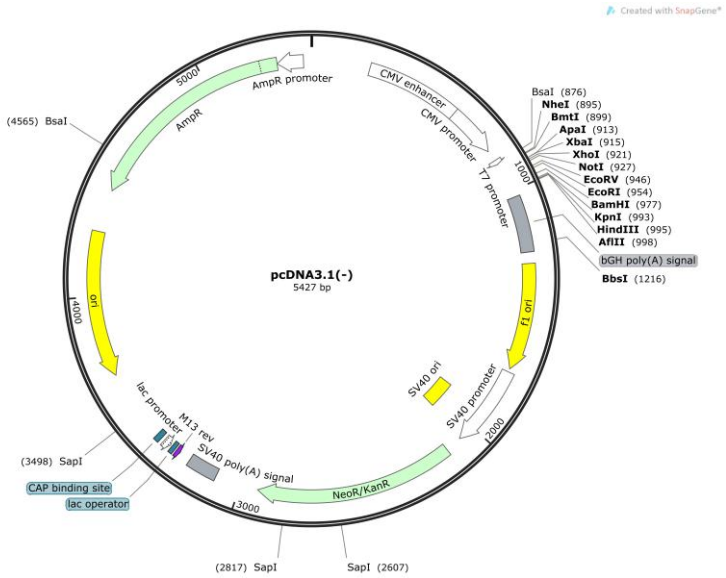

Figure 6.1 Vector map of pcDNA3.1(-) constructed from its original sequence, including endogenous TypeIIs restriction enzyme sites

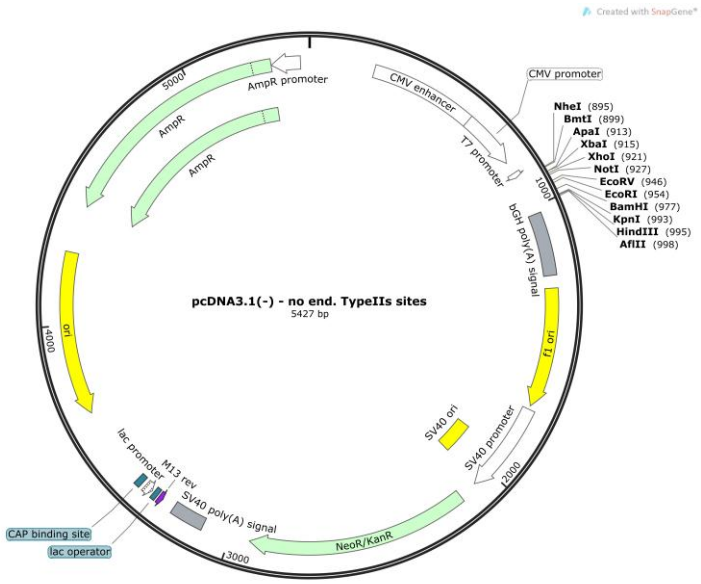

Figure 6.2 Vector map of pcDNA3.1(-) used for the cloning of the light-activated control GPCRs and light-activated human Class A oGPCRs, after the removal of the endogenous TypeIIs restriction enzymes sites

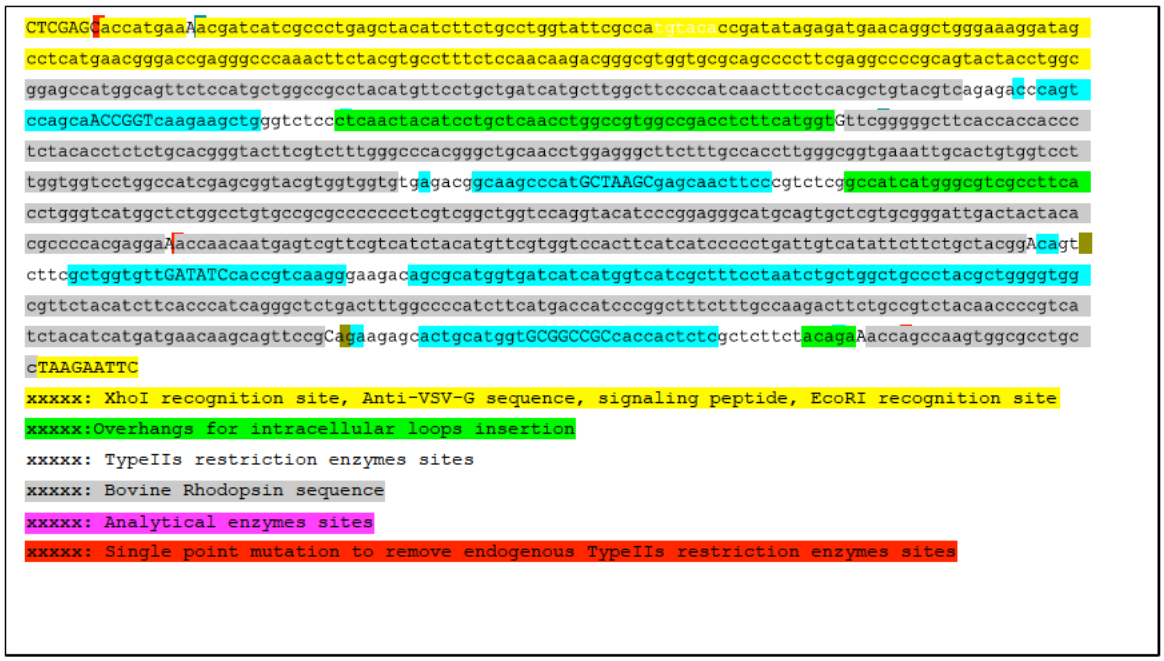

Figure 6.3 Sequence of the mother construct used for the creation of the light-activated control GPCRs and light-activated human Class A oGPCRs

Name	Sequence
Opto-GPR1	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVTGFKWKKTVLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAIE RYVVVIHPVLSHRHRTLKNAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFIIP LIVIFFCYGCLIFKVKKRSILISSRHFRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSAVYNPVIY IMMNKQFRARFRSSVAEILKYTLWEVSCSGTVSEQLRNSETKNLCLLETAQTETSQVAPA*
Opto-GPR3	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVIVGTPAFRAPLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAI ERYVVVYNALTYYSETTVTRAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFII PLIVIFFCYGQICRIVCRHAQQIALQRHLLPASHYVATRKGIRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIP AFFAKTSAVYNPVIYIMMNKQFRKVLWAVCCCCSSSKIPFRSRSPSDVTETSQVAPA*
Opto-GPR4	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVAYRQVQQRNELNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAI ERYVVVAHPLRFARLRRVKTAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFII PLIVIFFCYGGILRAVRGSVSTERQEKAKIKRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSAVYN PVIYIMMNKQFRSDVAKALHNLLRFLASDKPQEMANASLTLETPLTSKRNSTAKAMTGSWAATPPSQGDQVQLKMLPPAQTE TSQVAPA*
Opto-GPR6	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVIASTPALRTPLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAI ERYVVVYNALTYYSRRTLLGAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFII PLIVIFFCYGRICQVVWRHAHQIALQQHCLAPPHLAATRKGVRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIP AFFAKTSAVYNPVIYIMMNKQFRRALWLLLCGCFQSKVPFRSRSPSEVTETSQVAPA*
Opto-GPR12	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVIFHNPSLRAPLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAI ERYVVVYYALTYHSERTVTFAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFII PLIVIFFCYGQICKIVMRHAHQIALQHHFLATSHYVTTRKGVRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIP AFFAKTSAVYNPVIYIMMNKQFRKALCLICCGCIPSSLAQRARSPSDVTETSQVAPA*
Opto-GPR15	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVLHFKPGSRRLLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAI ERYVVVVWPVVSRKFRRTDCAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFII PLIVIFFCYGCIARKLCAHYQQSGKHNKKLKKSIRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSA VYNPVIYIMMNKQFRRAIVHCLCPCLKNYDFGSSTETSDSHLTKALSTFIHAEDFARRRKRSVSLTETSQVAPA*
Opto-GPR17	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVFIRDHKSGTPLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAI ERYVVVVHPVKSLKLRRPLYAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFII PLIVIFFCYGLIIRSLRQGLRVEKRLKTKAVRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSAVYN PVIYIMMNKQFRHALCNLLCGKRLKGPPPSFEGKTNESSLSAKSELTETSQVAPA*
Opto-GPR18	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVFSCTTKKRTTLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAI ERYVVVVQPKYAKELKNTCKAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFII PLIVIFFCYGVIIHNLLHGRTSKLKPKVKEKSIRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSAV YNPVIYIMMNKQFRARVISVMLYRNYLRSMRRKSFRSGSLRSLSNINSEMLTETSQVAPA*
Opto-GPR19	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVIHRSRRTQSTLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAI ERYVVVVYPLSFKVSREKAKAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFII PLIVIFFCYGKVIKYIWRIGTDGRTVRRTMNIVPRTKVKTIRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPA FFAKTSAVYNPVIYIMMNKQFRRGMKETFCMSSMKCYRSNAYTITTSSRMAKKNYVGISEIPSMAKTITKDSIYDSFDREAK

	EKKLAWPINSNPPNTFVTETSQVAPA*
Opto-GPR20	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVFCCRTRAKTPLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAI ERYVVVVRPEGSRRCRQPACAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFII PLIVIFFCYGRIMCALSRPGLLHQGRQRRVRAMRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSAV YNPVIYIMMNKQFRATVRGLFGQHGEREPSSGDVVSMHRSSKGSGRHHILSAGPHALTQALANGPEATETSQVAPA*
Opto-GPR21	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVFHCAPLLNHHTLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLA IERYVVVTKPLTYNTLVTPWRAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFI IPLIVIFFCYGNIFRICQQHTKDISERQARFSSQSGETGEVQACPDKRYARMVIIMVIAFLICWLPYAGVAFYIFTHQGSDF GPIFMTIPAFFAKTSAVYNPVIYIMMNKQFRRGLKRLSGAMCTSCASQTTANDPYTVRSKGPLNGCHITETSQVAPA*
Opto-GPR22	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVYCMKSNLINSVLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLA IERYVVVVKPANRILTMGRAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFIIP LIVIFFCYGKILQALNIRIGTRFSTGQKKKARKKKTISLTTQHEATDMSQSSGGRNVVFGVRTSVSVIIALRRAVKRHRERR ERQKRVFRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSAVYNPVIYIMMNKQFRKVLKSKMKKRVV SIVEADPLPNNAVIHNSWIDPKRNKKITFEDSEIREKCLVPQVVTDTETSQVAPA*
Opto-GPR25	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVLAGRRGPRRLLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAI ERYVVVVKLLEARPLRTPRCAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFII PLIVIFFCYGRISRRLRRPPHVGRARRNSLRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSAVYNP VIYIMMNKQFRARALDGACGRTGRLARRISSASSLSRDDSSVFRCRAQAANTASASWTETSQVAPA*
Opto-GPR26	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVLLHSADIRRQALNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLA IERYVVVVFPLSYRAKMRLRDAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFI IPLIVIFFCYGKVLKVARFHCKRIDVITMQTLVLLVDLHPSVRERCLEEQKRRRQRATRMVIIMVIAFLICWLPYAGVAFYI FTHQGSDFGPIFMTIPAFFAKTSAVYNPVIYIMMNKQFRKSCKEILNRLLHRRSIHSSGLTGDSHSQNILPVSETETSQVAP A*
Opto-GPR27	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVIVRERSLHRALNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAI ERYVVVAHHRFYAERLAGWPCAAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHF IIPLIVIFFCYGHDRRKMRPARLVPAVSHDWTFHGPGATGQAAANWTAGFGRGPTPPALVGIRPAGPGRGARRLLVLEEFKT EKRLCRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSAVYNPVIYIMMNKQFRDCFRAQFPCCQSPR TTQATHPCDLKGIGLTETSQVAPA*
Opto-GPR31	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVFLFRVRVWKPLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAI ERYVVVVHPRLKVNLLSPQAAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFII PLIVIFFCYGGIIRALQKRLREPEKQPKLQRAQRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSAV YNPVIYIMMNKQFRSSYRRVFHTLRGKGQAAEPPDFNPRDSYSTETSQVAPA*
Opto-GPR32	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVTVFRMARTVLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAIE RYVVVLYPVWALNHRTVQRAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFIP LIVIFFCYGLIRAKLLREGWVHANRPKRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSAVYNPVIY IMMNKQFREKFFQSLTSALARAFGEEEFLSSCPRGNAPRETETSQVAPA*
Opto-GPR33	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVLRFKMKQTVLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAIE RYVVVLHPVWSQQHRTPRWAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFIIP LIVIFFCYGRVASKVKERSLFKSSKPFRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSAVYNPVIY IMMNKQFRKVFKKSILALFESTFSEDSSVERTQTTETSQVAPA*
Opto-GPR34	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVFLGIHRKRNSLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAI ERYVVVNRSIQQRKAITTKQAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFII PLIVIFFCYGKIGKNLLRISKRRSKFPNSGKYATTARMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKT SAVYNPVIYIMMNKQFRKIMCQLLFRRFQGEPSRSESTSEFKPGYSLHDTSVAVKIQSSSKSTTETSQVAPA*
Opto-GPR35	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVFCCRMQQWTELNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAI ERYVVVRHPLRARGLRSPRQAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFII PLIVIFFCYGKVVTALAQRPPTDVGQAEATRKAARMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSA VYNPVIYIMMNKQFREASALAVAPSAKAHKSQDSLCVTLATETSQVAPA*
Opto-GPR37	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVVCHNYYMRSILNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAI ERYVVVTNVQMYYEMIENCSAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFII PLIVIFFCYGVTARKIRKAEKACTRGNKRQIQLESQMNRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFA KTSAVYNPVIYIMMNKQFRRAFMECCCCCCEECIQKSSTVTSDDNDNEYTTELELSPFSTIRREMSTFASVGTHCTETSQVA PA*
Opto-GPR37L1	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVVWHSYYLKSALNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAI ERYVVVTSTLPKVRPIERCQAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFII PLIVIFFCYGVTWRVRGPPGRKSECRASKHEQCESQLNRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFA KTSAVYNPVIYIMMNKQFRQAFLDCCCCCCCEECGGASEASAANGSDNKLKTEVSSSIYFHKPRESPPLLPLGTPCTETSQV APA*
Opto-GPR39	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVLQKKGYLQKELNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAI ERYVVVCHPFRYKAVSGPCQAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFII PLIVIFFCYGMMQVLMKSQKGSLAGGTRPPQLRKSESEESRTARRQTIRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGP IFMTIPAFFAKTSAVYNPVIYIMMNKQFRRVFVQVLCCRLSLQHANHEKRLRVHAHSTTDSARFVQRPLLFASRRQSSARRT EKIFLSTFQSEAEPQSKSQSLSLESLEPNSGAKPANSAAENGFQEHEVTETSQVAPA*
Opto-GPR42	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVFVGKLRCRPVALNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLA IERYVVVAHPLWYKTRPRLGQAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFI IPLIVIFFCYGRLVWILGRGGSHRRQRRVARMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSAVYNP VIYIMMNKQFRADFHELLRRLCGLWGQWQQESSMELKEQKGGEEQRADRPAERKTSEHSQGCGTGGQVACAENTETSQVAPA *

Opto-GPR45	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVVYQRPAMRSALNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAI ERYVVVVQRQDKLNPRRAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFIIPLI VIFFCYGCILNTVRKNAVRVHNQSDSLDLRQLTRAGLRRLQRQQQVSVDLSFKTKAFRMVIIMVIAFLICWLPYAGVAFYIF THQGSDFGPIFMTIPAFFAKTSAVYNPVIYIMMNKQFREACIELLPQTFQILPKVPERIRRRIQPSTVYVCNENQSAVTETS QVAPA*
Opto-GPR50	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVVTKNKKLRNSLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAI ERYVVVCHSLQYERIFSVRNAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFII PLIVIFFCYGRIWTKVLAARDPAGQNPDNQLAEVRNFRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAK TSAVYNPVIYIMMNKQFRREYWTIFHAMRHPIIFFSGLISDIREMQEARTLARARAHARDQAREQDRAHACPAVEETPMNVR NVPLPGDAAAGHPDRASGHPKPHSRSSSAYRKSASTHHKSVFSHSKAASGHLKPVSGHSKPASGHPKSATVYPKPASVHFKA DSVHFKGDSVHFKPDSVHFKPASSNPKPITGHHVSAGSHSKSAFSAATSHPKPTTGHIKPATSHAEPTTADYPKPATTSHPK PTAADNPELSASHCPEIPAIAHPVSDDSDLPESASSPAAGPTKPAASQLESDTIADLPDPTVVTTSTNDYHDVVVIDVEDDP DEMAVTETSQVAPA*
Opto-GPR52	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVFHCAPLLHHYTLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLA IERYVVVTKPLSYNQLVTPCRAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFI IPLIVIFFCYGHIFKICRQHTKEINDRRARFPSHEVDSSRETGHSPDRRYARMVIIMVIAFLICWLPYAGVAFYIFTHQGSD FGPIFMTIPAFFAKTSAVYNPVIYIMMNKQFRLGLRRLSETMCTSCMCVKDQEAQEPKPRKRANSCSITETSQVAPA*
Opto-GPR55	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVFSTFLKNRWPDYAALNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLV VLAIERYVVVRYPLLVSHLRSPRKAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVV HFIIPLIVIFFCYGRSIHILLGRRDHTQDWVQQKACIRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAK TSAVYNPVIYIMMNKQFRMNIRAHRPSRVQLVLQDTTISRGTETSQVAPA*
Opto-GPR61	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVIAKTPALRKLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAIE RYVVVVHPMRYEVRMTLGLAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFIIP LIVIFFCYGSMFRVARVAAMQHGPLPTWMETPRQRSESLSSRSTMVTSSGAPQTTPHRTFGGGKAARMVIIMVIAFLICWLP YAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSAVYNPVIYIMMNKQFRGELSKQFVCFFKPAPEEELRLPSREGSIEENFLQF LQGTGCPSESWVSRPLPSPKQEPPAVDFRIPGQIAEETSEFLEQQLTSDIIMSDSYLRPAASPRLESTETSQVAPA*
Opto-GPR62	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVVLRTPGLRDLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAIE RYVVVVHPLRPGSRPPAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFIIPLIV IFFCYGGIFVVARRAALRPPRPARGSRLHSDSLDSRLSILPPLRPRLPGGKAARMVIIMVIAFLICWLPYAGVAFYIFTHQG SDFGPIFMTIPAFFAKTSAVYNPVIYIMMNKQFRLALGRLSRRALPGPVRACTPQAWHPRALLQCLQRPPEGPAVGPSEAPE QTPELAGGRSPAYQGPPESSLSTETSQVAPA*
Opto-GPR63	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVVYQKAAMRSALNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAI ERYVVVVQRQDKLNPYRAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFIIPLI VIFFCYGGILNTLRHNALRIHSYPEGICLSQASKLGLMSLQRPFQMSIDMGFKTRAFRMVIIMVIAFLICWLPYAGVAFYIF THQGSDFGPIFMTIPAFFAKTSAVYNPVIYIMMNKQFRDACLDMMPKSFKFLPQLPGHTKRRIRPSAVYVCGEHRTVVTETS QVAPA*
Opto-GPR65	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVSFLQAKKESELNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAI ERYVVVVYPLKFFFLRTRRFAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFII PLIVIFFCYGKVYQAVRHNKATENKEKKRIIRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSAVYN PVIYIMMNKQFRYDMWNILKFCTGRCNTSQRQRKRILSVSTKDTMELEVLETETSQVAPA*
Opto-GPR68	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVGYLQIKARNELNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAI ERYVVVAHPFRFHQFRTLKAAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFII PLIVIFFCYGGILRAVRRSHGTQKSRKDQIQRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSAVYN PVIYIMMNKQFRRDLARLRGACLAFLTCSRTGRAREAYPLGAPEASGKSGAQGEEPELLTKLHPAFQTPNSPGSGGFPTGRL ATETSQVAPA*
Opto-GPR75	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVFFDPAFRKFRTNLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVL AIERYVVVLGKQPNRTASFPCAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFI IPLIVIFFCYGMIAQTLRKNAQVRKCPPVITVDASRPQPFMGVPVQGGGDPIQCAMPALYRNQNYNKLQHVQTRGYTKSPNQ LVTPAASRLQLVSAINLSTAKDSKAVRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSAVYNPVIYI MMNKQFRRKVLWCLQYIGLGFFCCKQKTRLRAMGKGNLEVNRNKSSHHETNSAYMLSPKPQKKFVDQACGPSHSKESMVSPK ISAGHQHCGQSSSTPINTRIEPYYSIYNSSPSQEESSPCNLQPVNSFGFANSYIAMHYHTTNDLVQEYDSTSAKQIPVPSVT ETSQVAPA*
Opto-GPR78	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVCAYSAELRTRALNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLA IERYVVVGFPLRYAGRLRPRYAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFI IPLIVIFFCYGQVHRVARRHCQRMDTVTMKALALLADLHPSVRQRCLIQQKRRRHRATRMVIIMVIAFLICWLPYAGVAFYI FTHQGSDFGPIFMTIPAFFAKTSAVYNPVIYIMMNKQFRQVLAGMVHRLLKRTPRPASTHDSSLDVAGMVHQLLKRTPRPAS THNGSVDTENDSCLQQTHTETSQVAPA*
Opto-GPR82	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVFLTKIGKKTSLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAI ERYVVVCYEKIFYGHLLKKFRQPNFAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFV VHFIIPLIVIFFCYGSFVSHLRKIRTCTSIMEKDLTYSSVRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAF FAKTSAVYNPVIYIMMNKQFRKTLYNLFTKSNSAHMQSYGTETSQVAPA*
Opto-GPR83	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVIFKNQRMHSALNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAI ERYVVVMHPLKPRISITKAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFIIPL IVIFFCYGRVAKKLWLCNMIGDVTTEQYFALRRKKKKTIRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFF AKTSAVYNPVIYIMMNKQFRIELKALLSMCQRPPKPQEDRPPSPVPSFRVAWTEKNDGQRAPLANNLLPTSQLQSGKTDLSS VEPIVTMSTETSQVAPA*
Opto-GPR84	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVLAIQPKLRTRLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAI ERYVVVAHPKLFPQVFSAKGAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFII PLIVIFFCYGLIHRQVKRAAQALDQYKLRQASIHSNHVARTDEAMPGRFQELDSRLASGGPSEGISSEPVSAATTQTLEGDS SEVGDQINSKRAKQMAEKSPPEASAKAQPIKGARRAPDSSSEFGKVTRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPI FMTIPAFFAKTSAVYNPVIYIMMNKQFRQAYGSILKRGPRSFHRLHTETSQVAPA*

Opto-GPR85	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVLVKDKTLHRALNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAI ERYVVVAHHRFYTKRLTFWTAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFII PLIVIFFCYGHDRRKMKPVQFVAAVSQNWTFHGPGASGQAAANWLAGFGRGPTPPTLLGIRQNANTTGRRRLLVLDEFKMEK RISRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSAVYNPVIYIMMNKQFRRCFSTTLLYCRKSRLP REPYCVITETSQVAPA*
Opto-GPR87	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVFFHIRNKTSLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAIE RYVVVVKPFGDSRMYSITFAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFIP LIVIFFCYGAISRYIHKSSRQFISQSSRKRKHNRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSAV YNPVIYIMMNKQFRRRLFKKSNIRTRSESIRSLQSVRRSEVRIYYDYTDVTETSQVAPA*
Opto-GPR88	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVVSSFRKLQTTLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAI ERYVVVRAPATYQALYQRRHAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFII PLIVIFFCYGGIVRRVRVSVKRVSVLNFHLLHQLPGCARMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFA KTSAVYNPVIYIMMNKQFRRSVRSVLPGVGDAAAAAVAATAVPAVSQAQLGTRAAGQHWTETSQVAPA*
Opto-GPR119	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVVLLLIHKNDGLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAI ERYVVVKQPFRYLKIMSGFVAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFII PLIVIFFCYGDMLKIASMHSQQIRKMEHAGAMAGGYRSPRTPSDFKALRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGP IFMTIPAFFAKTSAVYNPVIYIMMNKQFRLQLYHMALGVKKVLTSFLLFLSARNCGPERPRESSCHIVTISSSEFDGTETSQ VAPA*
Opto-GPR120	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVVARRRRRGALNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAIE RYVVVVHLQRGVRGPGRRAAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFIIP LIVIFFCYGKILQTSEHLLDARAVVTHSEITKASRKRLTVSLAYSESHQIRVSQQDFRLFRMVIIMVIAFLICWLPYAGVAF YIFTHQGSDFGPIFMTIPAFFAKTSAVYNPVIYIMMNKQFRWKKIFCCFWFPEKGAILTDTSVKRNDLSIISGTETSQVAPA *
Opto-GPR132	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVALLQVLQGNVLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAI ERYVVVVYALESRGRRRRRTAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFII PLIVIFFCYGRIFRSIKQSMGLSAAQKAKVKRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSAVYN PVIYIMMNKQFRQEVSRIHKGWKEWSMKTDVTRLTHSRDTEELQSPVALADHYTFSRPVHPPGSPCPAKRLIEESCTETSQV APA*
Opto-GPR135	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVIVKHRQLRTVLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAI ERYVVVVRPPREKIGRRRAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFIIPL IVIFFCYGHICKTVRLSDVRVRPVNTYARVLRFFSEVRTRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFF AKTSAVYNPVIYIMMNKQFRRNREEGYRTRNVDAFLPSQGPGLQARSRSRLRNRYANRLGACNRMSSSNPASGVAGDVAMWA RKNPVVLFCREGPPEPVTAVTKQPKSEAGDTSLTETSQVAPA*
Opto-GPR139	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVLSQLVARRQKLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAI ERYVVVCHPLKYHTVSYPARAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFII PLIVIFFCYGIIVYKLRRKSNFRLRGYSTGKTTRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSAV YNPVIYIMMNKQFRTMAAATLKAFFKCQKQPVQFYTNHNFSITSSPWISPANSHCIKMLVYQYDKNGKPIKVSPTETSQVAP A*
Opto-GPR141	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVLVKMNTRSVLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAIE RYVVVFKCKDKVEFYRKLAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFIIPL IVIFFCYGIIMLMVQKLRHSLLSHQEFWAQRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSAVYNP VIYIMMNKQFRQKIIGLWNCVLCRTETSQVAPA*
Opto-GPR142	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVLARLATRTRRPLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLA IERYVVVCHPLHHRAASSPGRAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFI IPLIVIFFCYGAIIHRLRRRGRSGLQPRVGKSTRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSAV YNPVIYIMMNKQFRATVRQVIHDAYLPCTLASQPEGMAAKPVMEPPGLPTGAEVTETSQVAPA*
Opto-GPR146	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVNLHSKASMTMLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAI ERYVVVALPRTYMASVYNAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFIIPL IVIFFCYGLLSRVRREDTPLDRDTGRLEPSAHRRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSAV YNPVIYIMMNKQFRSKLQRLMKKLPCGDRHCSPDHMGVQQVLATETSQVAPA*
Opto-GPR148	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVILRNQRLRQELNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAI ERYVVVIHPLRYLSFMSHGAAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFII PLIVIFFCYGRIYAEAKTSGIWGQGYSRARGTLRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSAV YNPVIYIMMNKQFRQLLGMVRGHLPSRRHQAIFTISTETSQVAPA*
Opto-GPR149	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVLLKMQNRTVLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAIE RYVVVHRGVGSQTASRRSGAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFIIP LIVIFFCYGEISRGASIPGTPPTAGRVVSLSPEDAPGPSLRRSGGCSPSSDTVFGPGAPAAAGAEACRRENRGTLYGTRSFT VSVAQKRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSAVYNPVIYIMMNKQFRLSFQKSYGIYKIA HEDYYDDDENSIFYHNLMNSECETTKDPQRDNRNIFNAIKVEISTTPSLDSSTQRGINKCTNTDITEAKQDSNNKKDAFSDK TGGDINYEETTFSEGPERRLSHEESQKPDLSDWEWCRSKSERTPRQRSGYALAIPLCAFQGTVSLHAPTGKTLSLSTYEVSA EGQKITPASKKIEVYRSKSVGHEPNSEDSSSTFVDTSVKIHLEVLEICDNEEALDTVSIISNISQSSTQVRSPSLRYSRKEN RFVSCDLGETASYSLFLPTSNPDGDINISIPDTVEAHRQNSKRQHQERDGYQEEIQLLNKAYRKREEESKGSTETSQVAPA*
Opto-GPR150	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVLCGGGGPWAGPKRRKLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSL VVLAIERYVVVRLPHGRPLPAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFII PLIVIFFCYGHLLSVWWRHRPQAPAAAAPWSASPGRAPAPSALPRAKVQSRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDF GPIFMTIPAFFAKTSAVYNPVIYIMMNKQFRRQLRKRLGSLCCAPQGGAEDEEGPRGHQALYRQRWPHPHYHHARREPLDEG GLRPPPPRPRPLPCSCESAFTETSQVAPA*
Opto-GPR151	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVLLHNAWKGKPSMLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVL AIERYVVVSDPAKQVSIHNYAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFII PLIVIFFCYGRAYDQCKKRGTKTQNLRNQIRSKQVTRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKT

	SAVYNPVIYIMMNKQFREGLKGVWKWMITKKPPTVSESQETPAGNSEGLPDKVPSPESPASIPEKEKPSSPSSGKGKTEKAE IPILPDVEQFWHERDTVPSVQDNDPIPWEHEDQETGEGVKTETSQVAPA*
Opto-GPR152	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVAGSQARHGAGTRLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVL AIERYVVVLCPHWYPGHRPVRLAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHF IIPLIVIFFCYGLTQATACRTCHRQQQPAACRGFARMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKTS AVYNPVIYIMMNKQFRTLLRSVLSSFAAALCEERPGSFTPTEPQTQLDSEGPTLPEPMAEAQSQMDPVAQPQVNPTLQPRSD PTAQPQLNPTAQPQSDPTAQPQLNLMAQPQSDSVAQPQADTNVQTPAPAASSVPSPCDEASPTPSSHPTPGALEDPATPPAS EGESPSSTPPEAAPGAGPTTETSQVAPA*
Opto-GPR153	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVVGAKQKKWKPLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAI ERYVVVCWPVNYRLSNAKKQAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFII PLIVIFFCYGTLAVQVGRQADRRAFTVPTIVVEDAQGKRRSSIDGSEPAKTSLQRMVIIMVIAFLICWLPYAGVAFYIFTHQ GSDFGPIFMTIPAFFAKTSAVYNPVIYIMMNKQFRADLKAVREKCMALMANDEESDDETSLEGGISPDLVLERSLDYGYGGD FVALDRMAKYEISALEGGLPQLYPLRPLQEDKMQYLQVPPTRRFSHDDADVWAAVPLPAFLPRWGSGEDLAALAHLVLPAGP ERRRASLLAFAEDAPPSRARRRSAESLLSLRPSALDSGPRGARDSPPGSPRRRPGPGPRSASASLLPDAFALTAFECEPQAL RRPPGPFPAAPAAPDGADPGEAPTPPSSAQRSPGPRPSAHSHAGSLRPGLSASWGEPGGLRAAGGGGSTSSFLSSPSESSGY ATLHSDSLGSASTETSQVAPA*
Opto-GPR160	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVTLGMRRKNTCQNLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVL AIERYVVVSKTTKLSFKCQKLFYFAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVV HFIIPLIVIFFCYGRITSYMNETILYFPFSSHSSYTVRSKKRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPA FFAKTSAVYNPVIYIMMNKQFRLKDIGLPLDPFVNWKCCFIPLTIPNLEQIEKPISIMICTETSQVAPA*
Opto-GPR161	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVLYKKSYLLTLLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAI ERYVVVLYPMVYPMKITGNRAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFII PLIVIFFCYGFIFRVARVKARKVHCGTVVIVEEDAQRTGRKNSSTSTSSSGSRRNAFQGVVYSANQCKALRMVIIMVIAFLI CWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSAVYNPVIYIMMNKQFRKELLGMCFGDRYYREPFVQRQRTSRLFSISN RITDLGLSPHLTALMAGGQPLGHSSSTGDTGFSCSQDSGTDMMLLEDYTSDDNPPSHCTCPPKRRSSVTFEDEVEQIKEAAK NSILHVKAEVHKSLDSYAASLAKAIEAEAKINLFGEEALPGVLVTARTVPGGGFGGRRGSRTLVSQRLQLQSIEEGDVLAAE QRTETSQVAPA*
Opto-GPR162	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVISAKQQKHKPLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAI ERYVVVRWPVNYRLSNAKKQAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFII PLIVIFFCYGTLWARPRRARQARRVGGGGGTKAGGPGALGTRPAFEVPAIVVEDARGKRRSSLDGSESAKTSLQRMVIIMVI AFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSAVYNPVIYIMMNKQFRADVRTVWEQCVAIMSEEDGDDDGGCDD YAEGRVCKVRFDANGATGPGSRDPAQVKLLPGRHMLFPPLERVHYLQVPLSRRLSHDETNIFSTPREPGSFLHKWSSSDDIR VLPAQSRALGGPPEYLGQRHRLEDEEDEEEAEGGGLASLRQFLESGVLGSGGGPPRGPGFFREEITTFIDETPLPSPTASPG HSPRRPRPLGLSPRRLSLGSPESRAVGLPLGLSAGRRCSLTGGEESARAWGGSWGPGNPIFPQLTLTETSQVAPA*
Opto-GPR171	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVFIQKNTNHRCLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAI ERYVVVTHSCKIYRIQEPGFAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFII PLIVIFFCYGLVIRQLYRNKDNENYPNVKKALRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSAVY NPVIYIMMNKQFRSKVTETFASPKETKAQKEKLRCENNATETSQVAPA*
Opto-GPR173	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVVLKERALHKALNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAI ERYVVVAHHRFYAKRMTLWTAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFII PLIVIFFCYGYRHRKMKPVQMVPAISQNWTFHGPGATGQAAANWIAGFGRGPMPPTLLGIRQNGHAASRRLLGMDEVKGEKQ LGRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSAVYNPVIYIMMNKQFRKCLRTHAPCWGTGGAPA PREPYCVMTETSQVAPA*
Opto-GPR174	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVFYGYMKETKRLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAI ERYVVVMYPFRFHDCKQKYDAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFII PLIVIFFCYGKTVLSLQDKYPMAQDLGEKQKALRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSAV YNPVIYIMMNKQFRRRLSRQDLHDSIQLHAKSFVSNHTASTMTPELCTETSQVAPA*
Opto-GPR176	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVTCRTTVFKSVLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAI ERYVVVLYPLERKISDAKAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFIIPL IVIFFCYGLIRRALSASQKKKVIIAALRTPQNTISIPYASQREAELHRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPI FMTIPAFFAKTSAVYNPVIYIMMNKQFRKCLIGTLVQLHHRYSRRNVVSTGSGMAEASLEPSIRSGSQLLEMFHIGQQQIFK PTEDEEESEAKYIGSADFQAKEIFSTCLEGEQGPQFAPSAPPLSTVDSVSQVAPAAPVEPETFPDKYSLQFGFGPFELPPQW LSETRNSKKRLLPPLGNTPEELIQTKVPKVGRVERKMSRNNKVSIFPKVDSTETSQVAPA*
Opto-GPR182	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVNWRGSGRAGLLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAI ERYVVVTSASPSWQRYQHRVRRAAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVH FIIPLIVIFFCYGLTACRLRQPGQPKSRRHCRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSAVYN PVIYIMMNKQFRGRLLNAVVHYLPKDQTKAGTCASSSSCSTQHSIIITKGDSQPAAAAPHPEPSLSFQAHHLLPNTSPISPT QPLTPSTETSQVAPA*
Opto-GPR183	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLG FPINFLTLYVIVQNRKKINSLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAI ERYVVVVHPLRYNKIKRIEHAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFII PLIVIFFCYGQICCKLFRTAKQNPLTEKSGVNKKALRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKT SAVYNPVIYIMMNKQFRRKVMRMLKRQVSVSISSAVKSAPEENSREMTETQMMIHSKSSNGKTETSQVAPA*

Table 6.1 Amino acid sequences of all light-activated human Class A oGPCRs created using the chimeric algorithm described in section 2.1

Name	Sequence
Opto-GPR1	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCACGGGGTTCAAGTGGAAGAAAACAGTCCTCAACTACATCCTGCTCAACCTGG CCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGGGCC CACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATCGAG CGGTACGTGGTGGTGATCCATCCTGTCTTATCTCATCGGCATCGAACCCTCAAGAACGCCATCATGGGCGTCGCCTTCACCT GGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTGCGG GATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATCCCC CTGATTGTCATATTCTTCTGCTACGGATGTCTCATCTTCAAGGTGAAGAAGCGAAGCATCCTGATCTCCAGTAGGCATTTCC GCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGGCGTTCTACATCTTCACCCATCA GGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGCCGTCTACAACCCCGTCATCTAC ATCATGATGAACAAGCAGTTCCGCGCTCGCTTCCGGTCCTCAGTTGCTGAGATACTCAAGTACACACTGTGGGAAGTCAGCT GTTCTGGCACAGTGAGTGAACAGCTCAGGAACTCAGAAACCAAGAATCTGTGTCTCCTGGAAACAGCTCAAACAGAAACCAG CCAAGTGGCGCCTGCCTAA
Opto-GPR3	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCATCGTGGGCACTCCTGCCTTCCGTGCCCCCCTCAACTACATCCTGCTCAACC TGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGG GCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATC GAGCGGTACGTGGTGGTGTACAATGCCCTCACCTACTATTCAGAGACAACAGTGACACGGGCCATCATGGGCGTCGCCTTCA CCTGGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTG CGGGATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATC CCCCTGATTGTCATATTCTTCTGCTACGGACAAATCTGCCGCATCGTCTGCCGCCATGCCCAGCAGATTGCCCTTCAGCGGC ACCTGCTGCCTGCCTCCCACTATGTGGCCACCCGCAAGGGCATTCGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTG CTGGCTGCCCTACGCTGGGGTGGCGTTCTACATCTTCACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCG GCTTTCTTTGCCAAGACTTCTGCCGTCTACAACCCCGTCATCTACATCATGATGAACAAGCAGTTCCGCAAAGTGCTGTGGG CTGTCTGCTGCTGCTGTTCCTCTTCCAAGATCCCCTTCCGATCCCGCTCCCCCAGTGATGTCACAGAAACCAGCCAAGTGGC GCCTGCCTAA
Opto-GPR4	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCGCCTACCGCCAGGTGCAACAGCGCAACGAGCTCAACTACATCCTGCTCAACC TGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGG GCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATC GAGCGGTACGTGGTGGTGGCCCACCCACTCCGCTTCGCCCGCCTGCGCCGCGTCAAGACCGCCATCATGGGCGTCGCCTTCA CCTGGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTG CGGGATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATC CCCCTGATTGTCATATTCTTCTGCTACGGAGGCATCCTGCGGGCCGTGCGGGGCAGCGTGTCCACCGAGCGCCAGGAGAAGG CCAAGATCAAGCGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGGCGTTCTACAT CTTCACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGCCGTCTACAAC CCCGTCATCTACATCATGATGAACAAGCAGTTCCGCAGCGATGTGGCCAAGGCCCTGCACAACCTGCTCCGCTTTCTGGCCA GCGACAAGCCCCAGGAGATGGCCAATGCCTCGCTCACCCTGGAAACCCCACTCACCTCCAAGAGGAACAGCACAGCCAAAGC CATGACTGGCAGCTGGGCGGCCACTCCGCCCTCCCAGGGGGACCAGGTGCAGCTGAAGATGCTGCCGCCAGCACAAACAGAA ACCAGCCAAGTGGCGCCTGCCTAA
Opto-GPR6	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCATCGCGTCCACTCCGGCGCTGCGCACGCCCCTCAACTACATCCTGCTCAACC TGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGG GCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATC GAGCGGTACGTGGTGGTGTATAACGCGCTCACCTATTACTCGCGCCGGACCCTGTTGGGCGCCATCATGGGCGTCGCCTTCA CCTGGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTG CGGGATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATC CCCCTGATTGTCATATTCTTCTGCTACGGACGCATCTGCCAGGTGGTCTGGCGCCACGCGCACCAGATCGCGCTGCAGCAGC ACTGCCTGGCGCCACCCCATCTCGCTGCCACCAGAAAGGGTGTGCGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTG CTGGCTGCCCTACGCTGGGGTGGCGTTCTACATCTTCACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCG GCTTTCTTTGCCAAGACTTCTGCCGTCTACAACCCCGTCATCTACATCATGATGAACAAGCAGTTCCGCCGCGCCCTGTGGC TCCTGCTCTGTGGCTGTTTCCAGTCCAAAGTGCCCTTTCGTTCCAGGTCCCCCAGCGAGGTCACAGAAACCAGCCAAGTGGC GCCTGCCTAA
Opto-GPR12	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCATCTTCCACAACCCCAGCCTGCGAGCACCCCTCAACTACATCCTGCTCAACC TGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGG GCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATC GAGCGGTACGTGGTGGTGTACTACGCTCTGACGTACCATTCGGAGAGGACGGTCACGTTTGCCATCATGGGCGTCGCCTTCA CCTGGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTG CGGGATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATC CCCCTGATTGTCATATTCTTCTGCTACGGACAGATCTGTAAGATTGTGATGAGGCACGCCCATCAGATAGCCCTGCAGCACC ACTTCCTGGCCACGTCGCACTATGTGACCACCCGGAAAGGGGTGCGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTG CTGGCTGCCCTACGCTGGGGTGGCGTTCTACATCTTCACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCG GCTTTCTTTGCCAAGACTTCTGCCGTCTACAACCCCGTCATCTACATCATGATGAACAAGCAGTTCCGCAAAGCGCTCTGTC TCATTTGCTGCGGCTGCATCCCGTCCAGTCTCGCCCAGAGAGCGCGCTCGCCCAGTGATGTGACAGAAACCAGCCAAGTGGC GCCTGCCTAA
Opto-GPR15	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCTTGCATTTCAAACCCGGCAGCCGAAGGCTGCTCAACTACATCCTGCTCAACC TGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGG GCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATC GAGCGGTACGTGGTGGTGGTGTGGCCAGTCGTATCCAGGAAATTCAGAAGGACAGACTGTGCCATCATGGGCGTCGCCTTCA

	CCTGGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTG CGGGATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATC CCCCTGATTGTCATATTCTTCTGCTACGGATGCATTGCAAGGAAGCTGTGTGCCCATTACCAGCAATCAGGAAAGCACAACA AAAAGCTGAAGAAATCTATACGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGGC GTTCTACATCTTCACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGCC GTCTACAACCCCGTCATCTACATCATGATGAACAAGCAGTTCCGCCGGGCCATTGTCCACTGCTTGTGCCCTTGCCTGAAAA ACTATGACTTTGGGAGTAGCACTGAGACATCAGATAGTCACCTCACTAAGGCTCTCTCCACCTTCATTCATGCAGAAGATTT TGCCAGGAGGAGGAAGAGGTCTGTGTCACTCACAGAAACCAGCCAAGTGGCGCCTGCCTAA
Opto-GPR17	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCTTTATCCGAGATCACAAGTCCGGGACCCCGCTCAACTACATCCTGCTCAACC TGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGG GCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATC GAGCGGTACGTGGTGGTGGTGCACCCGGTCAAGTCCCTCAAGCTCCGCAGGCCCCTCTACGCCATCATGGGCGTCGCCTTCA CCTGGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTG CGGGATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATC CCCCTGATTGTCATATTCTTCTGCTACGGACTGATCATCCGCAGCCTGCGGCAGGGCCTGCGTGTGGAGAAGCGCCTCAAGA CCAAGGCAGTGCGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGGCGTTCTACAT CTTCACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGCCGTCTACAAC CCCGTCATCTACATCATGATGAACAAGCAGTTCCGCCACGCCCTGTGCAACTTGCTCTGTGGCAAAAGGCTCAAGGGCCCGC CCCCCAGCTTCGAAGGGAAAACCAACGAGAGCTCGCTGAGTGCCAAGTCAGAGCTGACAGAAACCAGCCAAGTGGCGCCTGC CTAA
Opto-GPR18	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCTTTAGTTGTACCACCAAGAAGAGAACCACGCTCAACTACATCCTGCTCAACC TGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGG GCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATC GAGCGGTACGTGGTGGTGGTACAGCCGAAGTACGCCAAAGAACTTAAAAACACGTGCAAAGCCATCATGGGCGTCGCCTTCA CCTGGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTG CGGGATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATC CCCCTGATTGTCATATTCTTCTGCTACGGAGTCATTATTCATAATCTCCTTCACGGCAGGACGTCTAAGCTGAAACCCAAAG TCAAGGAGAAGTCCATACGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGGCGTT CTACATCTTCACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGCCGTC TACAACCCCGTCATCTACATCATGATGAACAAGCAGTTCCGCGCTCGAGTCATTAGTGTCATGCTATACCGTAATTACCTTC GAAGCATGCGCAGAAAAAGTTTCCGATCTGGTAGTCTACGGTCACTAAGCAATATAAACAGTGAAATGTTAACAGAAACCAG CCAAGTGGCGCCTGCCTAA
Opto-GPR19	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCATCCATAGGAGTAGGAGGACTCAGTCTACCCTCAACTACATCCTGCTCAACC TGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGG GCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATC GAGCGGTACGTGGTGGTGGTCTATCCTCTGAGCTTCAAGGTGTCCAGAGAAAAAGCCAAGGCCATCATGGGCGTCGCCTTCA CCTGGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTG CGGGATTGACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATC CCCCTGATTGTCATATTCTTCTGCTACGGAAAGGTCATAAAATATATTTGGAGAATAGGCACAGATGGCCGAACGGTGAGGA GGACAATGAACATTGTCCCTCGGACAAAAGTGAAAACTATCCGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTG GCTGCCCTACGCTGGGGTGGCGTTCTACATCTTCACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCT TTCTTTGCCAAGACTTCTGCCGTCTACAACCCCGTCATCTACATCATGATGAACAAGCAGTTCCGCAGAGGGATGAAAGAGA CTTTTTGCATGTCCTCTATGAAATGTTACCGAAGCAATGCCTATACTATCACAACAAGTTCAAGGATGGCCAAAAAAAACTA CGTTGGCATTTCAGAAATCCCTTCCATGGCCAAAACTATTACCAAAGACTCGATCTATGACTCATTTGACAGAGAAGCCAAG GAAAAAAAGCTTGCTTGGCCCATTAACTCAAATCCACCAAATACTTTTGTCACAGAAACCAGCCAAGTGGCGCCTGCCTAA
Opto-GPR20	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCTTTTGCTGCCGCACCCGGGCCAAGACACCCCTCAACTACATCCTGCTCAACC TGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGG GCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATC GAGCGGTACGTGGTGGTGGTGCGGCCCGAAGGCTCCCGCCGCTGCCGCCAGCCTGCCTGTGCCATCATGGGCGTCGCCTTCA CCTGGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTG CGGGATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATC CCCCTGATTGTCATATTCTTCTGCTACGGACGCATCATGTGTGCACTGTCGCGGCCGGGTCTGCTCCACCAGGGTCGCCAGC GCCGCGTGCGGGCCATGCGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGGCGTT CTACATCTTCACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGCCGTC TACAACCCCGTCATCTACATCATGATGAACAAGCAGTTCCGCGCCACCGTCCGAGGCCTCTTCGGCCAGCACGGAGAGCGTG AGCCCAGCAGCGGTGACGTGGTCAGCATGCACAGGAGCTCCAAGGGCTCAGGCCGTCATCACATCCTCAGTGCCGGCCCTCA CGCCCTCACCCAGGCCCTGGCTAATGGGCCCGAGGCTACAGAAACCAGCCAAGTGGCGCCTGCCTAA
Opto-GPR21	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCTTTCACTGTGCACCTTTGTTGAACCATCACACTCTCAACTACATCCTGCTCA ACCTGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTT TGGGCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCC ATCGAGCGGTACGTGGTGGTGACTAAACCTTTAACCTATAATACTCTGGTTACACCCTGGAGAGCCATCATGGGCGTCGCCT TCACCTGGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTC GTGCGGGATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATC ATCCCCCTGATTGTCATATTCTTCTGCTACGGAAACATCTTCCGCATCTGCCAACAGCACACAAAGGACATCAGCGAAAGGC AAGCCCGCTTCAGCAGCCAGAGTGGGGAGACTGGGGAAGTGCAGGCCTGTCCTGATAAGCGCTATGCCCGCATGGTGATCAT CATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGGCGTTCTACATCTTCACCCATCAGGGCTCTGACTTT GGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGCCGTCTACAACCCCGTCATCTACATCATGATGAACA AGCAGTTCCGCAGAGGACTAAAGCGCCTCTCAGGGGCTATGTGTACTTCTTGTGCAAGTCAGACTACAGCCAACGACCCTTA CACAGTTAGAAGCAAAGGCCCTCTTAATGGATGTCATATCACAGAAACCAGCCAAGTGGCGCCTGCCTAA

Opto-GPR22	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCTACTGCATGAAATCCAACTTAATCAACTCTGTCCTCAACTACATCCTGCTCA ACCTGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTT TGGGCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCC ATCGAGCGGTACGTGGTGGTGGTAAAACCTGCAAACCGAATTCTGACAATGGGCAGAGCCATCATGGGCGTCGCCTTCACCT GGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTGCGG GATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATCCCC CTGATTGTCATATTCTTCTGCTACGGAAAAATACTTCAGGCTCTTAATATTCGAATAGGCACAAGATTTTCAACAGGGCAGA AGAAGAAAGCAAGAAAGAAAAAGACAATTTCTCTAACCACACAACATGAGGCTACAGACATGTCACAAAGCAGTGGTGGGAG AAATGTAGTCTTTGGTGTAAGAACTTCAGTTTCTGTAATAATTGCCCTCCGGCGAGCTGTGAAACGACACCGTGAACGACGA GAAAGACAAAAGAGAGTGTTCCGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGG CGTTCTACATCTTCACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGC CGTCTACAACCCCGTCATCTACATCATGATGAACAAGCAGTTCCGCAAGGTCTTGAAAAGTAAAATGAAAAAGCGAGTTGTT TCTATAGTAGAAGCTGATCCCCTGCCTAATAATGCTGTAATACACAACTCTTGGATAGATCCTAAAAGAAACAAAAAAATTA CCTTTGAAGATAGTGAAATAAGAGAAAAATGTTTAGTGCCTCAGGTTGTCACAGACACAGAAACCAGCCAAGTGGCGCCTGC CTAA
Opto-GPR25	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCCTGCTGCACAGCGCGGACATCCGCCGCCAGGCGCTCAACTACATCCTGCTCA ACCTGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTT TGGGCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCC ATCGAGCGGTACGTGGTGGTGGTGTTCCCGTTGAGCTACCGGGCCAAGATGCGCCTCCGCGACGCCATCATGGGCGTCGCCT TCACCTGGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTC GTGCGGGATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATC ATCCCCCTGATTGTCATATTCTTCTGCTACGGAAAGGTGCTCAAGGTGGCCCGCTTCCATTGCAAGCGCATCGACGTGATCA CCATGCAGACGCTGGTGCTGCTGGTGGACCTGCACCCCAGTGTGCGGGAACGCTGTCTGGAGGAGCAGAAGCGGAGGCGACA GCGAGCCACCCGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGGCGTTCTACATC TTCACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGCCGTCTACAACC CCGTCATCTACATCATGATGAACAAGCAGTTCCGCAAAAGCTGCAAGGAGATTCTGAACAGGCTCCTGCACAGACGCTCCAT CCACTCCTCTGGCCTCACAGGCGACTCTCACAGCCAGAACATTCTGCCGGTGTCTGAGACAGAAAC CAGCCAAGTGGCGCCT GCCTAA
Opto-GPR26	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCATCGTGCGGGAGCGCAGCCTGCACCGCGCCCTCAACTACATCCTGCTCAACC TGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGG GCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATC GAGCGGTACGTGGTGGTGGCGCACCACCGCTTCTATGCAGAGCGCCTGGCCGGCTGGCCGTGCGCCGCCATCATGGGCGTCG CCTTCACCTGGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTG CTCGTGCGGGATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTC ATCATCCCCCTGATTGTCATATTCTTCTGCTACGGACACGACCGCCGCAAGATGCGGCCCGCGCGCCTGGTGCCCGCCGTCA GCCACGACTGGACCTTCCACGGCCCGGGCGCCACCGGCCAGGCCGCCGCCAACTGGACGGCGGGCTTCGGCCGCGGGCCCAC GCCGCCCGCGCTTGTGGGCATCCGGCCCGCAGGGCCGGGCCGCGGCGCGCCGCCTCCTCGTGCTGGAAGAATTCAAGACG GAGAAGAGGCTGTGCCGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGGCGTTCT ACATCTTCACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGCCGTCTA CAACCCCGTCATCTACATCATGATGAACAAGCAGTTCCGCGACTGCTTCAGGGCCCAGTTCCCCTGCT GCCAGAGCCCCCGG ACCACCCAGGCGACCCATCCCTGCGACCTGAAAGGCATTGGTTTAACAGAAACCAGCCAAGTGGCGCCTGCCTAA
Opto-GPR27	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTTTCTGTTCCGGGTCAGGGTGTGGAAGCCGCTCAACTACATCCTGCTCAACC TGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGG GCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATC GAGCGGTACGTGGTGGTGGTCCACCCTCGGCTTAAGGTCAACCTGCTGTCTCCTCAGGCGGCCATCATGGGCGTCGCCTTCA CCTGGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTG CGGGATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATC CCCCTGATTGTCATATTCTTCTGCTACGGAGGCATCATCAGGGCTCTCCAGAAAAGACTCCGGGAGCCTGAGAAACAGCCCA AGCTTCAGCGGGCCCAGCGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGG CGTT CTACATCTTCACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGCCGTC TACAACCCCGTCATCTACATCATGATGAACAAGCAGTTCCGCAGCTCCTATCGGAGGGTGTTCCACACCCTCCGAGGCAAAG GGCAGGCAGCAGAGCCCCCAGATTTCAACCCCAGAGACTCCTATTCCACAGAAACCAGCCAAGTGGCGCCTGCCTAA
Opto-GPR31	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCTTTCTGTTCCGGGTCAGGGTGTGGAAGCCGCTCAACTACATCCTGCTCAACC TGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGG GCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATC GAGCGGTACGTGGTGGTGGTCCACCCTCGGCTTAAGGTCAACCTGCTGTCTCCTCAGGCGGCCATCATGGGCGTCGCCTTCA CCTGGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTG CGGGATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATC CCCCTGATTGTCATATTCTTCTGCTACGGAGGCATCATCAGGGCTCTCCAGAAAAGACTCCGGGAGCCTGAGAAACAGCCCA AGCTTCAGCGGGCCCAGCGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGG CGTT CTACATCTTCACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGCCGTC TACAACCCCGTCATCTACATCATGATGAACAAGCAGTTCCGCAGCTCCTATCGGAGGGTGTTCCACACCCTCCGAGGCAAAG GGCAGGCAGCAGAGCCCCCAGATTTCAACCCCAGAGACTCCTATTCCACAGAAACCAGCCAAGTGGCGCCTGCCTAA
Opto-GPR32	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCACTGTGTTCCGTATGGCACGCACGGTGCTCAACTACATCCTGCTCAACCTGG CCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGGGCC CACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATCGAG

	CGGTACGTGGTGGTGCTCTACCCCGTCTGGGCCCTGAACCACCGCACTGTGCAGCGGGCCATCATGGGCGTCGCCTTCACCT GGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTGCGG GATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATCCCC CTGATTGTCATATTCTTCTGCTACGGACTCATCCGGGCCAAGCTCTTGCGGGAGGGCTGGGTCCATGCCAACCGGCCCAAGC GCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGGCGTTCTACATCTTCACCCATCA GGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGCCGTCTACAACCCCGTCATCTAC ATCATGATGAACAAGCAGTTCCGCGAAAAGTTTTTCCAGTCTTTGACTTCTGCCCTGGCGAGGGCGTTTGGAGAGGAGGAGT TTCTGTCATCCTGTCCCCGTGGCAACGCCCCCCGGGAAACAGAAACCAGCCAAGTGGCGCCTGCCTAA
Opto-GPR33	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCCTAAGATTCAAGATGAAACAGACTGTCCTCAACTACATCCTGCTCAACCTGG CCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGGGCC CACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATCGAG CGGTACGTGGTGGTGCTTCACCCAGTGTGGTCCCAGCAGCACCGAACCCCGCGCTGGGCCATCATGGGCGTCGCCTTCACCT GGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTGCGG GATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATCCCC CTGATTGTCATATTCTTCTGCTACGGAAGAGTAGCCAGCAAGGTGAAAGAGAGGAGCCTGTTTAAATCCAGCAAGCCCTTCC GCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGGCGTTCTACATCTTCACCCATCA GGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGCCGTCTACAACCCCGTCATCTAC ATCATGATGAACAAGCAGTTCCGCAAGGTGTTCAAGAAGTCCATTCTTGCTCTGTTTGAGTCAACATTTAGTGAAGATTCTT CTGTAGAAAGGACACAAACCACAGAAACCAGCCAAGTGGCGCCTGCCTAA
Opto-GPR34	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCC GCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCCTTTTCTGGGTATTCACCGTAAAAGAAATTCCCTCAACTACATCCTGCTCAACC TGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGG GCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATC GAGCGGTACGTGGTGGTGAATCGGTCTATACAGCAACGGAAGGCAATAACAACCAAACAAGCCATCATGGGCGTCGCCTTCA CCTGGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTG CGGGATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGT GGTCCACTTCATCATC CCCCTGATTGTCATATTCTTCTGCTACGGAAAGATTGGGAAGAATCTATTGAGGATTTCTAAAAGGAGGTCAAAATTTCCTA ATTCTGGTAAATATGCCACTACAGCTCGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGG GGTGGCGTTCTACATCTTCACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTT GCCAAGACT TCTGCCGTCTACAACCCCGTCATCTACATCATGATGAACAAGCAGTTCCGCAAAATAATGTGCCAACTTCTTTTTAGACGAT TTCAAGGTGAACCAAGTAGGAGTGAAAGCACTTCAGAATTTAAACCAGGATACTCCCTGCATGATACATCTGTGGCAGTGAA AATACAGTCTAGTTCTAAAAGTACTACAGAAACCAGCCAAGTGGCGCCTGCCTAA
Opto-GPR35	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCTTTTGCTGCCGCATGCAGCAGTGGACGGAACTCAACTACATCCTGCTCAACC TGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGG GCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATC GAGCGGTACGTGGTGGTGCGGCACCCGCTGCGTGCCCGCGGGCTGCGGTCCCCCAGGCAGGCCATCATGGGCGTCGCCTTCA CCTGGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTG CGGGATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATC CCCCTGATTGTCATATTCTTCTGCTACGGAAAGGTGGTGACTGCCCTGGCCCAGAGGCCACCCACCGACGTGGGGCAGGCAG AGGCCACCCGCAAGGCTGCCCGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGGC GTTCTACATCTTCACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGCC GTCTACAACCCCGTCATCTACATCATGATGAACAAGCAGTTCCGCGAGGCGTCTGCACTGGCCGTGGCTCCCAGTGCTAAGG CCCACAAAAGCCAGGACTCTCTGTGCGTGACCCTCGCCACAGAAACCAGCCAAGTGGCGCCTGCCTAA
Opto-GPR37	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCGTGTGGCCACAACTACTACATGCGGAGCATCCTCAACTACATCCTGCTCAACC TGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGG GCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATC GAGCGGTACGTGGTGGTGACCAACGTACAGATGTACTACGAAATGATCGAAAACTGTTCCGCCATCATGGGCGTCGCCTTCA CCTGGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTG CGGGATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATC CCCCTGATTGTCATATTCTTCTGCTACGGAGTGACTGCGAGGAAAATCCGCAAAGCAGAGAAAGCCTGTACCCGAGGGAATA AACGGCAGATTCAACTAGAGAGTCAGATGAACCGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTA CGCTGGGGTGGCGTTCTACATCTTCACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCC AAGACTTCTGCCGTCTACAACCCCGTCATCTACATCATGATGAACAAGCAGTTCCGCCGGGCCTTCATGGAGTGCTGCTGCT GTTGCTGTGAGGAATGCATTCAGAAGTCCTCAACGGTGACCAGTGATGACAATGACAACGAGTACACCACGGAACTCGAACT CTCGCCTTTCAGTACCATACGCCGTGAAATGTCCACTTTTGCTTCTGTCGGAACTCATTGCACAGAAACCAGCCAAGTGGCG CCTGCCTAA
Opto-GPR37L1	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCGTGTGGCACAGCTACTACCTGAAGAGTGCCCTCAACTACATCCTGCTCAACC TGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGG GCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATC GAGCGGTACGTGGTGGTGACCAGCACCCTGCCCAAGGTGAGGCCCATCGAGCGGTGCCAAGCCATCATGGGCGTCGCCTTCA CCTGGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTG CGGGATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATC CCCCTGATTGTCATATTCTTCTGCTACGGAGTGACATGGCGGGTGCGAGGCCCTCCAGGGAGGAAGTCAGAGTGCAGGGCCA GCAAGCACGAGCAGTGTGAGAGCCAGCTCAACCGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTA CGCTGGGGTGGCGTTCTACATCTTCACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCC AAGACTTCTGCCGTCTACAACCCCGTCATCTACATCATGATGAACAAGCAGTTCCGCCAGGCCTTCCTGGACTGCTGCTGCT GCTGCTGCTGTGAGGAGTGCGGCGGGGCTTCGGAGGCCTCTGCTGCCAATGGGTCGGACAACAAGCTCAAGACCGAGGTGTC CTCTTCCATCTACTTCCACAAGCCCAGGGAGTCACCCCCACTCCTGCCCCTGGGCACACCTTGCACAGAAACCAGCCAAGTG GCGCCTGCCTAA
Opto-GPR39	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT

	CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCCTGCAGAAGAAAGGATACTTGCAGAAGGAGCTCAACTACATCCTGCTCAACC TGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGG GCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATC GAGCGGTACGTGGTGGTGTGTCACCCCTTCAGGTACAAGGCTGTGTCGGGACCTTGCCAGGCCATCATGGGCGTCGCCTTCA CCTGGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTG CGGGATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATC CCCCTGATTGTCATATTCTTCTGCTACGGAATGATGCAGGTGCTCATGAAAAGCCAGAAGGGCTCGCTGGCCGGGGGCACGC GGCCTCCGCAGCTGAGGAAGTCCGAGAGCGAAGAGAGCAGGACCGCCAGGAGGCAGACCATCCGCATGGTGATCATCATGGT CATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGGCGTTCTACATCTTCACCCATCAGGGCTCTGACTTTGGCCCC ATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGCCGTCTACAACCCCGTCATCTACATCATGATGAACAAGCAGT TCCGCCGGGTGTTCGTGCAGGTGCTGTGCTGCCGCCTGTCGCTGCAGCACGCCAACCACGAGAAGCGCCTGCGCGTACATGC GCACTCCACCACCGACAGCGCCCGCTTTGTGCAGCGCCCGTTGCTGTTCGCGTCCCGGCGCCAGTCCTCTCGCAAGGAGAACT GAGAAGATTTTCTTAAGCACTTTTCAGAGCGAGGCCGAGCCCCAGTCTAAGTCCCAGTCATTGAGTCTCGAGTCACTAGAGC CCAACTCAGGCGCGAAACCAGCCAATTCTGCTGCAGAGAATGGTTTTCAGGAGCATGAAGTTACAGAAACCAGCCAAGTGGC GCCTGCCTAA
Opto-GPR42	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCTTTGTGGGCAAGCTGCGGTGCCGCCCGGTGGCCCTCAACTACATCCTGCTCA ACCTGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTT TGGGCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCC ATCGAGCGGTACGTGGTGGTGGCCCACCCACTGTGGTACAAGACCCGGCCGAGGCTGGGGCAGGCCATCATGGGCGTCGCCT TCACCTGGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTC GTGCGGGATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATC ATCCCCCTGATTGTCATATTCTTCTGCTACGGACGCCTGGTGTGGATCCTCGGCAGAGGGGGCAGCCACCGCCGGCAGAGGA GGGTGGCGCGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGGCGTTCTACATCTT CACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGCCGTCTACAACCCC GTCATCTACATCATGATGAACAAGCAGTTCCGCGCCGACTTTCATGAGCTGCTGAGGAGGTTGTGTGGGCTCTGGGGCCAGT GGCAGCAGGAGAGCAGCATGGAGCTGAAGGAGCAGAAGGGAGGGGAGGAGCAGAGAGCGGACCGACCAGCTGAAAGAAAGAC CAGTGAACACTCACAGGGCTGTGGAACTGGTGGCCAGGTGGCCTGTGCTGAAAACACAGAAACCAGCCAAGTGGCGCCTGCC TAA
Opto-GPR45	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCGTGTACCAGAGGCCGGCTATGCGCTCGGCCCTCAACTACATCCTGCTCAACC TGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGG GCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATC GAGCGGTACGTGGTGGTGGTCCAGCGCCAGGACAAGCTGAACCCGCGCAGGGCCATCATGGGCGTCGCCTTCACCTGGGTCA TGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTGCGGGATTGA CTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATCCCCCTGATT GTCATATTCTTCTGCTACGGATGCATCCTCAACACGGTCCGCAAGAACGCCGTGCGCGTGCACAACCAGTCGGACAGCCTGG ACCTGCGGCAGCTCACCAGGGCGGGGCCTGCGGCGCCTGCAGCGGCAGCAACAGGTCAGCGTGGACTTGAGCTTCAAGACCAA GGCCTTCCGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGGCGTTCTACATCTTC ACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGCCGTCTACAACCCCG TCATCTACATCATGATGAACAAGCAGTTCCGCGAGGCCTGCATAGAGTTGCTGCCCCAGACCTTCCAAATCCTCCCCAAAGT GCCTGAGCGGATCCGAAGGAGAATCCAGCCAAGCACAGTCTACGTGTGCAATGAAAACCAGTCTGCGGTTACAGAAACCAGC CAAGTGGCGCCTGCCTAA
Opto-GPR50	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCGTGACGAAGAACAAGAAGCTCCGGAATTCTCTCAACTACATCCTGCTCAACC TGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGG GCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATC GAGCGGTACGTGGTGGTGTGCCACAGCCTCCAGTACGAACGGATCTTCAGTGTGCGCAATGCCATCATGGGCGTCGCCTTCA CCTGGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTG CGGGATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATC CCCCTGATTGTCATATTCTTCTGCTACGGAAGGATCTGGACCAAAGTGCTGGCGGCCCGTGACCCTGCAGGGCAGAATCCTG ACAACCAACTTGCTGAGGTTCGCAATTTTCGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGC TGGGGTGGCGTTCTACATCTTCACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAG ACTTCTGCCGTCTACAACCCCGTCATCTACATCATGATGAACAAGCAGTTCCGCAGAGAATACTGGACCATCTTCCATGCTA TGCGGCACCCTATCATATTCTTCTCTGGCCTCATCAGTGATATTCGTGAGATGCAGGAGGCCCGTACCCTGGCCCGCGCCCG TGCCCATGCTCGCGACCAAGCTCGTGAACAAGACCGTGCCCATGCCTGTCCTGCTGTGGAGGAAACCCCGATGAATGTCCGG AATGTTCCATTACCTGGTGATGCTGCAGCTGGCCACCCCGACCGTGCCTCTGGCCACCCTAAGCCCCATTCCAGATCCTCCT CTGCCTATCGCAAATCTGCCTCTACCCACCACAAGTCTGTCTTTAGCCACTCCAAGGCTGCCTCTGGTCACCTCAAGCCTGT CTCTGGCCACTCCAAGCCTGCCTCTGGTCACCCCAAGTCTGCCACTGTCTACCCTAAGCCTGCCTCTGTCCATTTCAAGGCT GACTCTGTCCATTTCAAGGGTGACTCTGTCCATTTCAAGCCTGACTCTGTTCATTTCAAGCCTGCTTCCAGCAACCCCAAGC CCATCACTGGCCACCATGTCTCTGCTGGCAGCCACTCCAAGTCTGCCTTCAGTGCTGCCACCAGCCACCCTAAACCCACCAC TGGCCACATCAAGCCAGCTACCAGCCATGCAGAGCCCACCACTGCTGACTATCCCAAGCCTGCCACTACCAGCCACCCTAAG CCCACTGCTGCTGACAACCCTGAGCTCTCTGCCTCCCATTGCCCCGAGATCCCTGCCATTGCCCACCCTGTGTCTGACGACA GTGACCTCCCTGAGTCGGCCTCTAGCCCTGCCGCTGGGCCCACCAAGCCTGCTGCCAGCCAGCTGGAGTCTGACACCATCGC TGACCTTCCTGACCCTACTGTAGTCACTACCAGTACCAATGATTACCATGATGTCGTGGTTATTGATGTTGAAGATGATCCT GATGAAATGGCTGTGACAGAAACCAGCCAAGTGGCGCCTGCCTAA
Opto-GPR52	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCTTTCATTGTGCTCCACTGTTACATCATTATACTCTCAACTACATCCTGCTCA ACCTGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTT TGGGCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCC ATCGAGCGGTACGTGGTGGTGACCAAGCCTCTTTCCTACAATCAACTGGTCACCCCTTGTCGCGCCATCATGGGCGTCGCCT TCACCTGGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTC GTGCGGGATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATC ATCCCCCTGATTGTCATATTCTTCTGCTACGGACACATTTTCAAAATTTGCCGTCAGCACACCAAAGAGATAAATGACCGAA GGGCCCGATTCCCTAGTCATGAGGTAGATTCTTCCAGAGAGACTGGACACAGCCCTGACCGTCGCTACGCCCGCATGGTGAT CATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGGCGTTCTACATCTTCACCCATCAGGGCTCTGAC

	TTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGCCGTCTACAACCCCGTCATCTACATCATGATGA ACAAGCAGTTCCGCCTAGGCCTCCGAAGGCTGTCTGAGACAATGTGCACATCCTGTATGTGTGTGAAGGATCAGGAAGCACA AGAACCCAAACCTAGGAAACGGGCTAATTCTTGCTCCATTACAGAAACCAGCCAAGTGGCGCCTGCCTAA
Opto-GPR55	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCTTTAGCACCTTCCTTAAGAACAGGTGGCCCGATTATGCTGCCCTCAACTACA TCCTGCTCAACCTGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTA CTTCGTCTTTGGGCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTG GTCCTGGCCATCGAGCGGTACGTGGTGGTGCGTTACCCGCTACTGGTGAGCCACCTCCGGTCCCCAGGAAGGCCATCATGG GCGTCGCCTTCACCTGGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCAT GCAGTGCTCGTGCGGGATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTC CACTTCATCATCCCCCTGATTGTCATATTCTTCTGCTACGGAAGGAGCATCCACATCCTGCTGGGCCGCCGAGATCACACCC AGGACTGGGTGCAGCAGAAAGCCTGCATCCGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGC TGGGGTGGCGTTCTACATCTTCACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAG ACTTCTGCCGTCTACAACCCCGTCATCTACATCATGATGAACAAGCAGTTCCGCATGAACATCAGGGCCCACCGGCCTTCCA GGGTCCAGCTGGTCCTGCAGGACACCACGATCTCCCGGGGGCACAGAAACCAGCCAAGTGGCGCCTGCCTAA
Opto-GPR61	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCGTGCTGCGCACGCCGGGACTGCGCGACCTCAACTACATCCTGCTCAACCTGG CCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGGGCC CACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATCGAG CGGTACGTGGTGGTGGTGCACCCGCTGCGGCCAGGCTCGCGCCCGCCGGCCATCATGGGCGTCGCCTTCACCTGGGTCATGG CTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTGCGGGATTGACTA CTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATCCCCCTGATTGTC ATATTCTTCTGCTACGGAGGCATCTTCGTGGTGGCGCGTCGCGCTGCCCTGAGGCCCCCACGGCCGGCGCGCGGGTCCCGAC TCCACTCGGACTCTCTGGATAGCCGCCTTTCCATCTTGCCGCCGCTCCGGCCTCGCCTGCCCGGGGGCAAGGCGGCCCGCAT GGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGGCGTTCTACATCTTCACCCATCAGGGC TCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGCCGTCTACAACCCCGTCATCTACATCA TGATGAACAAGCAGTTCCGCTTGGCACTGGGCCGCCTCTCTCGCCGTGCACTGCCTGGACCTGTGCGGGCCTGCACTCCGCA AGCCTGGCACCCGCGGGCACTCTTGCAATGCCTCCAGAGGCCCCCAGAGGGCCCTGCCGTAGGCCCTTCTGAGGCTCCAGAA CAGACCCCCGAGTTGGCAGGAGGGCGGAGCCCCGCATACCAGGGGCCACCTGAGAGTTCTCTCTCCACAGAAACCAGCCAAG TGGCGCCTGCCTAA
Opto-GPR62	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCGTTTACCAAAAAGCTGCCATGAGGTCTGCACTCAACTACATCCTGCTCAACC TGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGG GCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATC GAGCGGTACGTGGTGGTGCAGGAGGCAGGATAAGCTAAACCCATATAGAGCCATCATGGGCGTCGCCTTCACCTGGGTCA TGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTGCGGGATTGA CTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATCCCCCTGATT GTCATATTCTTCTGCTACGGAGGCATACTCAACACCCTTCGGCACAATGCCTTGAGGATCCATAGCTACCCTGAAGGTATAT GCCTCAGCCAGGCCAGCAAACTGGGCCTCATGAGTCTGCAGAGGCCTTTCCAGATGAGCATTGACATGGGCTTTAAAACACG TGCCTTCCGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGGCGTTCTACATCTTC ACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGCCGTCTACAACCCCG TCATCTACATCATGATGAACAAGCAGTTCCGCGATGCTTGCCTGGACATGATGCCTAAGTCCTTCAAGTTTTTGCCGCAGCT CCCTGGTCACACAAAGCGACGGATACGTCCTAGTGCTGTCTATGTGTGTGGGGAACATCGGACGGTGGTGACAGAAACCAGC CAAGTGGCGCCTGCCTAA
Opto-GPR63	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCAGCTTCCTGCAAGCAAAGAAGGAAAGTGAACTCAACTACATCCTGCTCAACC TGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGG GCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATC GAGCGGTACGTGGTGGTGGTCTACCCTTTGAAGTTTTTTTTCCTAAGGACAAGAAGATTTGCCATCATGGGCGTCGCCTTCA CCTGGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTG CGGGATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATC CCCCTGATTGTCATATTCTTCTGCTACGGAAAAGTCTACCAAGCTGTGCGGCACAATAAAGCCACGGAAAACAAGGAAAAGA AGAGAATCATACGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGGCGTTCTACAT CTTCACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGCCGTCTACAAC CCCGTCATCTACATCATGATGAACAAGCAGTTCCGCTATGATATGTGGAATATATTAAAATTCTGCACTGGGAGGTGTAATA CATCACAAAGACAAAGAAAACGCATACTTTCTGTGTCTACAAAAGATACTATGGAATTAGAGGTCCTTGAGACAGAAACCAG CCAAGTGGCGCCTGCCTAA
Opto-GPR68	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT

	CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCGGCTACCTGCAGATCAAGGCCCGGAACGAGCTCAACTACATCCTGCTCAACC TGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGG GCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATC GAGCGGTACGTGGTGGTGGCCCATCCCTTCCGCTTCCACCAGTTCCGGACCCTGAAGGCGGCCATCATGGGCGTCGCCTTCA CCTGGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTG CGGGATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATC CCCCTGATTGTCATATTCTTCTGCTACGGAGGCATCCTGCGCGCCGTGCGCCGGAGCCACGGCACCCAGAAAAGCCGCAAGG ACCAGATCCAGCGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGGCGTTCTACAT CTTCACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGCCGTCTACAAC CCCGTCATCTACATCATGATGAACAAGCAGTTCCGCCGGGACCTGGCCCGCCTCCGCGGGGCCTGCCTGGCCTTCCTCACCT GCTCCAGGACCGGCCGGGCCAGGGAGGCCTACCCGCTGGGTGCCCCCGAGGCCTCCGGGAAAAGCGGGGCCCAGGGTGAGGA GCCCGAGCTGTTGACCAAGCTCCACCCGGCCTTCCAGACCCCTAACTCGCCAGGGTCGGGCGGGTTCCCCACGGGCAGGTTG GCCACAGAAACCAGCCAAGTGGCGCCTGCCTAA
Opto-GPR75	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCTTTTTCGATCCAGCCTTCAGGAAATTCAGAACCAACCTCAACTACATCCTGC TCAACCTGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGT CTTTGGGCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTG GCCATCGAGCGGTACGTGGTGGTGTTGGGGAAACAGCCTAATCGCACGGCCTCCTTTCCCTGCGCCATCATGGGCGTCGCCT TCACCTGGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTC GTGCGGGATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATC ATCCCCCTGATTGTCATATTCTTCTGCTACGGAATGATTGCTCAGACCCTGCGGAAGAACGCTCAAGTCAGAAAGTGCCCCC CTGTAATCACAGTCGATGCTTCCAGACCACAGCCTTTCATGGGGGTCCCTGTGCAGGGAGGTGGAGATCCCATCCAGTGTGC CATGCCGGCTCTGTATAGGAACCAGAATTACAACAAACTGCAGCACGTTCAGACCCGTGGATATACCAAGAGTCCCAACCAA CTGGTCACCCCTGCAGCAAGCCGACTCCAGCTCGTATCAGCCATCAACCTCTCCACTGCCAAGGATTCCAAAGCCGTGCGCA TGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGGCGTTCTACATCTTCACCCATCAGGG CTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGCCGTCTACAACCCCGTCATCTACATC ATGATGAACAAGCAGTTCCGCAGGAAAGTGCTCTGGTGCCTCCAATACATAGGCCTGGGTTTTTTCTGCTGCAAACAAAAGA CTCGACTTCGAGCCATGGGAAAAGGGAACCTCGAAGTCAACAGAAACAAATCCTCCCATCATGAAACAAACTCTGCCTACAT GTTATCTCCAAAGCCACAGAAGAAATTTGTGGACCAGGCTTGTGGCCCAAGTCATTCAAAAGAAAGTATGGTGAGTCCCAAG ATCTCTGCTGGACATCAACACTGTGGTCAGAGCAGCTCGACCCCCATCAACACTCGGATTGAACCTTACTACAGCATCTATA ACAGCAGCCCTTCCCAGGAGGAGAGCAGCCCATGTAACTTACAGCCAGTAAACTCTTTTGGATTTGCCAATTCATATATTGC CATGCATTATCACACCACTAATGACTTAGTGCAGGAATATGACAGCACTTCAGCCAAGCAGATTCCAGTCCCCTCCGTTACA GAAACCAGCCAAGTGGCGCCTGCCTAA
Opto-GPR78	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCTGCGCCTACAGCGCAGAGCTCCGCACTCGAGCCCTCAACTACATCCTGCTCA ACCTGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTT TGGGCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCC ATCGAGCGGTACGTGGTGGTGGGCTTCCCACTGCGCTACGCCGGACGCCTGCGACCGCGCTATGCCATCATGGGCGTCGCCT TCACCTGGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTC GTGCGGGATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATC ATCCCCCTGATTGTCATATTCTTCTGCTACGGACAGGTGCACCGGGTGGCACGCAGACACTGCCAGCGCATGGACACCGTCA CCATGAAGGCGCTCGCGCTGCTCGCCGACCTGCACCCCAGTGTGCGGCAGCGCTGCCTCATCCAGCAGAAGCGGCGCCGCCA CCGCGCCACCCGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGGCGTTCTACATC TTCACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGCCGTCTACAACC CCGTCATCTACATCATGATGAACAAGCAGTTCCGCCAAGTCCTGGCCGGCATGGTGCACCGGCTGCTGAAGAGAACCCCGCG CCCAGCATCCACCCATGACAGCTCTCTGGATGTGGCCGGCATGGTGCACCAGCTGCTGAAGAGAACCCCGCGCCCAGCGTCC ACCCACAACGGCTCTGTGGACACAGAGAATGATTCCTGCCTGCAGCAGACACACACAGAAACCAGCCAAGTGGCGCCTGCCT AA
Opto-GPR82	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCTTTTTAACAAAAATAGGTAAAAAAACATCACTCAACTACATCCTGCTCAACC TGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGG GCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATC GAGCGGTACGTGGTGGTGTGCTATGAGAAAATATTTTATGGCCATTTACTGAAAAAATTTCGCCAGCCCAACTTTGCCATCA TGGGCGTCGCCTTCACCTGGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGG CATGCAGTGCTCGTGCGGGATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTG GTCCACTTCATCATCCCCCTGATTGTCATATTCTTCTGCTACGGATCTTTTGTAAGCCATCTGAGAAAAATAAGAACCTGTA CGTCCATTATGGAGAAAGATTTGACTTACAGTTCTGTGCGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCT GCCCTACGCTGGGGTGGCGTTCTACATCTTCACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTC TTTGCCAAGACTTCTGCCGTCTACAACCCCGTCATCTACATCATGATGAACAAGCAGTTCCGCAAAACACTATATAATCTCT TTACAAAGTCTAATTCAGCACATATGCAATCATATGGTACAGAAACCAGCCAAGTGGCGCCTGCCTAA
Opto-GPR83	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCATCTTCAAGAACCAGCGAATGCACTCGGCCCTCAACTACATCCTGCTCAACC TGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGG GCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATC GAGCGGTACGTGGTGGTGATGCACCCCTTGAAACCCCGGATCTCAATCACAAAGGCCATCATGGGCGTCGCCTTCACCTGGG TCATGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTGCGGGAT TGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATCCCCCTG ATTGTCATATTCTTCTGCTACGGACGTGTGGCCAAGAAACTGTGGCTGTGTAATATGATTGGCGATGTGACCACAGAGCAGT ACTTTGCCCTGCGGCGCAAAAAGAAGAAAACCATCCGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCC CTACGCTGGGGTGGCGTTCTACATCTTCACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTT GCCAAGACTTCTGCCGTCTACAACCCCGTCATCTACATCATGATGAACAAGCAGTTCCGCATTGAGCTAAAGGCATTACTGA GCATGTGTCAAAGACCTCCCAAGCCTCAGGAGGACAGGCCACCCTCCCCAGTTCCTTCCTTCAGGGTGGCCTGGACAGAGAA GAATGATGGCCAGAGGGCTCCCCTTGCCAATAACCTCCTGCCCACCTCCCAACTCCAGTCTGGGAAAACAGACCTGTCATCT GTGGAACCCATTGTGACGATGAGTACAGAAACCAGCCAAGTGGCGCCTGCCTAA
Opto-GPR84	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT

	CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCTTGGCCATCCAGCCCAAGCTCCGTACCCGACTCAACTACATCCTGCTCAACC TGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGG GCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATC GAGCGGTACGTGGTGGTGGCCCACCCTAAGCTTTTTCCCCAAGTTTTCAGTGCCAAGGGGGCCATCATGGGCGTCGCCTTCA CCTGGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTG CGGGATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATC CCCCTGATTGTCATATTCTTCTGCTACGGACTCATCCACCGCCAGGTCAAACGAGCAGCACAGGCACTGGACCAATACAAGT TGCGACAGGCAAGCATCCACTCCAACCATGTGGCCAGGACTGATGAGGCCATGCCTGGTCGTTTCCAGGAGCTGGACAGCAG GTTAGCATCAGGAGGACCCAGTGAGGGGATTTCATCTGAGCCAGTCAGTGCTGCCACCACCCAGACCCTGGAAGGGGACTCA TCAGAAGTGGGAGATCAGATCAACAGCAAGAGAGCAAAGCAGATGGCAGAGAAAAGCCCTCCAGAAGCATCTGCCAAAGCCC AGCCAATTAAAGGAGCCAGAAGGGCTCCGGATTCTTCATCGGAATTTGGGAAGGTGACTCGCATGGTGATCATCATGGTCAT CGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGGCGTTCTACATCTTCACCCATCAGGGCTCTGACTTTGGCCCCATC TTCATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGCCGTCTACAACCCCGTCATCTACATCATGATGAACAAGCAGTTCC GCCAAGCATATGGCTCCATTTTAAAAAGAGGGCCCCGGAGTTTCCATAGGCTCCATACAGAAACCAGCCAAGTGGCGCCTGC CTAA
Opto-GPR85	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCCTAGTGAAAGATAAGACCTTGCATAGAGCACTCAACTACATCCTGCTCAACC TGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGG GCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATC GAGCGGTACGTGGTGGTGGCCCATCACCGCTTCTATACAAAGAGGCTGACCTTTTGGACGGCCATCATGGGCGTCGCCTTCA CCTGGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTG CGGGATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATC CCCCTGATTGTCATATTCTTCTGCTACGGACACGATCGAAGAAAAATGAAGCCAGTTCGTAGCAGCAGTCAGCCAGA ACTGGACTTTTCATGGTCCTGGAGCCAGTGGCCAGGCAGCTGCCAATTGGCTAGCAGGATTTGGAAGGGGTCCCACACCACC CACCTTGCTGGGCATCAGGCAAAATGCAAACACCACAGGCAGAAGAAGGCTATTGGTCTTAGACGAGTTCAAAATGGAGAAA AGAATCAGCCGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGGCGTTCTACATCT TCACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGCCGTCTACAACCC CGTCATCTACATCATGATGAACAAGCAGTTCCGCCGCTGTTTCAGCACAACCCTTCTTTACTGCAGAAAATCCAGGTTACCA AGGGAACCTTACTGTGTTATAACAGAAACCAGCCAAGTGGCGCCTGCCTAA
Opto-GPR87	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCTTTTTCCACATTAGGAATAAAACCAGCCTCAACTACATCCTGCTCAACCTGG CCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGGGCC CACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATCGAG CGGTACGTGGTGGTGGTCAAGCCATTTGGGGACTCTCGGATGTACAGCATAACCTTCGCCATCATGGGCGTCGCCTTCACCT GGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTGCGG GATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATCCCC CTGATTGTCATATTCTTCTGCTACGGAGCCATATCCAGGTACATCCACAAATCCAGCAGGCAATTCATAAGTCAGTCAAGCC GAAAGCGAAAACATAACCGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGGCGTT CTACATCTTCACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGCCGTC TACAACCCCGTCATCTACATCATGATGAACAAGCAGTTCCGCAGAAGGCTGTTCAAAAAATCAAATATCAGAACCAGGAGTG AAAGCATCAGATCACTGCAAAGTGTGAGAAGATCGGAAGTTCGCATATATTATGATTACACTGATGTGACAGAAACCAGCCA AGTGGCGCCTGCCTAA
Opto-GPR88	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCGTGCTGCTGTTGATCCACAAGAATGATGGGCTCAACTACATCCTGCTCAACC TGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGG GCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATC GAGCGGTACGTGGTGGTGAAGCAGCCCTTCCGCTACTTGAAGATCATGAGTGGGTTCGTGGCCATCATGGGCGTCGCCTTCA CCTGGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTG CGGGATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATC CCCCTGATTGTCATATTCTTCTGCTACGGAGACATGCTCAAGATTGCCTCCATGCACAGCCAGCAGATTCGAAAGATGGAAC ATGCAGGAGCCATGGCTGGAGGTTATCGATCCCCACGGACTCCCAGCGACTTCAAAGCTCTCCGCATGGTGATCATCATGGT CATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGGCGTTCTACATCTTCACCCATCAGGGCTCTGACTTTGGCCCC ATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGCCGTCTACAACCCCGTCATCTACATCATGATGAACAAGCAGT TCCGCCTGCAGCTCTACCACATGGCCCTAGGAGTGAAGAAGGTGCTCACCTCATTCCTCCTCTTTCTCTCGGCCAGGAATTG TGGCCCAGAGAGGCCCAGGGAAAGTTCCTGTCACATCGTCACTATCTCCAGCTCAGAGTTTGATGGCACAGAAACCAGCCAA GTGGCGCCTGCCTAA
Opto-GPR119	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCGCGCTGCCGACGACGCCGCGGCGCGCTCAACTACATCCTGCTCAACCTGG CCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGGGCC CACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATCGAG CGGTACGTGGTGGTGGTGCACCTGCAGCGCGGCGTGCGGGGTCCTGGGCGGCGGGCGGCCATCATGGGCGTCGCCTTCACCT

	GGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTGCGG GATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATCCCC CTGATTGTCATATTCTTCTGCTACGGAAAAATTTTACAGACCTCGGAACACCTCCTGGATGCAAGAGCTGTCGTGACTCACA GTGAGATCACAAAGGCATCAAGGAAGAGGCTCACGGTAAGCCTGGCCTACTCGGAGAGCCACCAGATCCGCGTGTCCCAGCA GGACTTCCGGCTGTTCCGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGGCGTTC TACATCTTCACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGCCGTCT ACAACCCCGTCATCTACATCATGATGAACAAGCAGTTCCGCTGGAAGAAAATTTTTTGCTGCTTCTGGTTCCCAGAAAAGGG AGCCATTTTAACAGACACATCTGTCAAAAGAAATGACTTGTCGATTATTTCTGGCACAGAAACCAGCCAAGTGGCGCCTGCC TAA
Opto-GPR132	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCGCGCTGCTGCAGGTACTGCAGGGCAACGTGCTCAACTACATCCTGCTCAACC TGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGG GCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATC GAGCGGTACGTGGTGGTGGTGTACGCGCTGGAGAGTCGGGGCCGCCGCCGCCGGAGGACCGCCATCATGGGCGTCGCCTTCA CCTGGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTG CGGGATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATC CCCCTGATTGTCATATTCTTCTGCTACGGACGGATTTTCAGGAGCATCAAGCAGAGCATGGGCTTAAGCGCTGCCCAGAAGG CCAAGGTGAAGCGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGGCGTTCTACAT CTTCACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGCCGTCTACAAC CCCGTCATCTACATCATGATGAACAAGCAGTTCCGCCAAGAAGTGTCCAGAATCCATAAGGGGTGGAAAGAGTGGTCCATGA AAACAGACGTCACCAGGCTCACCCACAGCAGGGACACCGAGGAGCTGCAGTCGCCCGTGGCCCTTGCAGACCACTACACCTT CTCCAGGCCCGTGCACCCACCAGGGTCACCATGCCCTGCAAAGAGGCTGATTGAGGAGTCCTGTACAGAAACCAGCCAAGTG GCGCCTGCCTAA
Opto-GPR135	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCATTGTGAAGCACCGGCAGCTCCGCACCGTCCTCAACTACATCCTGCTCAACC TGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGG GCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATC GAGCGGTACGTGGTGGTGGTGCGCCCGCCGCGGGAGAAGATCGGCCGCCGCCGCGCCATCATGGGCGTCGCCTTCACCTGGG TCATGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTGCGGGAT TGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATCCCCCTG ATTGTCATATTCTTCTGCTACGGACACATCTGCAAGACGGTGCGCCTGTCGGACGTGCGCGTGCGGCCGGTGAACACCTACG CGCGCGTGCTGCGCTTCTTCAGCGAGGTGCGCACGCGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCC CTACGCTGGGGTGGCGTTCTACATCTTCACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTT GCCAAGACTTCTGCCGTCTACAACCCCGTCATCTACATCATGATGAACAAGCAGTTCCGCCGCAACCGCGAGGAGGGCTACC GGACTAGGAATGTGGACGCTTTCCTGCCCAGCCAGGGCCCGGGTCTGCAAGCCAGAAGCCGCAGTCGCCTTCGAAACCGCTA TGCCAACCGGCTGGGGGCCTGCAACAGGATGTCCTCTTCCAACCCGGCCAGCGGAGTGGCAGGGGACGTGGCCATGTGGGCC CGCAAAAATCCAGTTGTACTTTTCTGCCGAGAGGGACCACCAGAGCCGGTGACGGCAGTGACCAAACAGCCTAAATCCGAAG CTGGGGATACCAGCCTCACAGAAACCAGCCAAGTGGCGCCTGCCTAA
Opto-GPR139	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCCTCTCCCAGCTGGTGGCAAGAAGGCAGAAGCTCAACTACATCCTGCTCAACC TGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGG GCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATC GAGCGGTACGTGGTGGTGTGCCACCCGCTCAAGTACCACACGGTGTCATACCCAGCCCGCGCCATCATGGGCGTCGCCTTCA CCTGGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTG CGGGATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATC CCCCTGATTGTCATATTCTTCTGCTACGGAATCATTGTGTACAAGCTCAGGAGGAAAAGCAATTTTCGGCTCCGTGGCTACT CCACGGGGAAAACCACCCGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGGCGTT CTACATCTTCACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGCCGTC TACAACCCCGTCATCTACATCATGATGAACAAGCAGTTCCGCACCATGGCAGCCGCCACGCTCAAGGCTTTCTTCAAGTGCC AGAAGCAACCTGTACAGTTCTACACCAATCATAACTTTTCCATAACAAGTAGCCCCTGGATCTCGCCGGCAAACTCACACTG CATCAAGATGCTGGTGTACCAGTATGACAAAAATGGAAAACCTATAAAAGTATCCCCGACAGAAACCAGCCAAGTGGCGCCT GCCTAA
Opto-GPR141	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCCTGGTGAAAATGAACACCCGGTCAGTGCTCAACTACATCCTGCTCAACCTGG CCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGGGCC CACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATCGAG CGGTACGTGGTGGTGTTCAAGTGCAAAGACAAAGTGGAATTCTACAGAAAACTGGCCATCATGGGCGTCGCCTTCACCTGGG TCATGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTGCGGGAT TGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATCCCCCTG ATTGTCATATTCTTCTGCTACGGAATCATTATGTTGATGGTGCAGAAGCTACGCCACTCTTTACTATCCCACCAGGAGTTCT GGGCACAGCGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGGCGTTCTACATCTT CACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGCCGTCTACAACCCC GTCATCTACATCATGATGAACAAGCAGTTCCGCCAAAAGATAATTGGCTTATGGAATTGTGTTTTGTGCCGTAC AGAAACCA GCCAAGTGGCGCCTGCCTAA
Opto-GPR142	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCCTGGCGCGCCTTGCCACCAGGACCAGGAGGCCCCTCAACTACATCCTGCTCA ACCTGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTT TGGGCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCC ATCGAGCGGTACGTGGTGGTGTGCCACCCCCTGCACCATCGGGCCGCCTCGTCCCCAGGCCGGGCCATCATGGGCGTCGCCT TCACCTGGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTC GTGCGGGATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATC ATCCCCCTGATTGTCATATTCTTCTGCTACGGAGCCATCATCCACCGGCTACGGAGGAGGGGCCGGAGTGGGCTGCAGCCCC GGGTGGGCAAGAGCACACGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGGCGTT CTACATCTTCACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGCCGTC TACAACCCCGTCATCTACATCATGATGAACAAGCAGTTCCGCGCCACTGTCCGACAGGTCATCCACGATGCCTACCTGCCCT

	GCACTTTGGCATCACAGCCAGAGGGCATGGCGGCGAAGCCTGTGATGGAGCCTCCGGGACTCCCCACAGGGGCAGAAGTGAC AGAAACCAGCCAAGTGGCGCCTGCCTAA
Opto-GPR146	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCAACCTACACAGCAAGGCCAGCATGACCATGCTCAACTACATCCTGCTCAACC TGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGG GCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATC GAGCGGTACGTGGTGGTGGCACTGCCGCGGACCTACATGGCCAGCGTGTACAACGCCATCATGGGCGTCGCCTTCACCTGGG TCATGGCTCTGGCCTGTGCCGCGCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTGCGGGAT TGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATCCCCCTG ATTGTCATATTCTTCTGCTACGGACTACTCTCCCGCGTCCGCAGGGAGGACACGCCCCTGGACCGGGACACGGGCCGGCTGG AGCCCTCGGCACACAGGCGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGGCGTT CTACATCTTCACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGCCGTC TACAACCCCGTCATCTACATCATGATGAACAAGCAGTTCCGCAGCAAGCTCCAACGGCTGATGAAAAAGCTGCCCTGCGGGG ACCGGCACTGCTCCCCGGACCACATGGGGGTGCAGCAGGTGCTGGCGACAGAAACCAGCCAAGTGGCGCCTGCCTAA
Opto-GPR148	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCCTGCTGAAAATGCAGAACAGAACTGTTCTCAACTACATCCTGCTCAACCTGG CCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGGGCC CACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATCGAG CGGTACGTGGTGGTGCACAGAGGTGTGGGGAGCCAGACAGCCTCCAGAAGATCGGGCGCCATCATGGGCGTCGCCTTCACCT GGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTGCGG GATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATCCCC CTGATTGTCATATTCTTCTGCTACGGAGAAATTTCCCGTGGAGCTTCAATTCCTGGGACCCCTCCTACTGCGGGGAGAGTGG TTTCCCTGTCCCCAGAGGATGCTCCAGGCCCGAGTCTGCGGCGCTCTGGGGGATGCTCTCCGAGCTCCGACACCGTGTTCGG ACCGGGTGCGCCCGCTGCCGCTGGGGCTGAAGCCTGCAGGCGTGAGAACCGGGGGACTCTCTATGGCACCAGGAGCTTCACC GTGAGCGTAGCGCAGAAGCGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGGCGT TCTACATCTTCACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGCCGT CTACAACCCCGTCATCTACATCATGATGAACAAGCAGTTCCGCCTATCATTCCAAAAAAGTTATGGGATTTATAAAATAGCA CATGAAGATTACTATGATGATGATGAAAATTCCATATTCTATCACAACCTGATGAACTCTGAGTGTGAAACTACAAAAGACC CTCAGAGAGACAACCGTAACATCTTCAATGCTATAAAAGTAGAAATCAGCACCACGCCCTCTCTGGACAGCTCCACACAAAG AGGCATCAACAAATGCACAAATACTGATATTACAGAAGCTAAACAGGATTCCAACAACAAAAAGGATGCGTTTTCTGACAAA ACAGGAGGTGATATTAACTATGAAGAAACTACCTTTTCTGAAGGGCCAGAAAGAAGGCTGTCTCATGAAGAGAGTCAGAAAC CAGATCTTTCAGACTGGGAGTGGTGTAGGAGTAAATCAGAAAGAACCCCTCGTCAGCGTTCCGGTTATGCCCTTGCCATTCC CTTGTGTGCATTCCAGGGGACTGTGTCTCTCCATGCACCTACAGGGAAAACCCTATCTCTTTCTACCTATGAGGTAAGCGCA GAAGGGCAAAAAATAACTCCAGCCTCTAAGAAAATAGAAGTCTATCGATCCAAAAGTGTTGGCCATGAACCAAACTCAGAAG ATTCTTCATCCACGTTTGTGGACACCAGTGTGAAAATACACTTGGAGGTTCTTGAAATTTGTGATAATGAAGAGGCCTTGGA CACTGTGTCAATCATTAGTAACATCAGTCAGTCCTCCACACAAGTCAGATCTCCATCCCTACGTTACTCCAGGAAAGAAAAC AGATTTGTTTCATGTGACCTAGGGGAAACAGCCTCATACTCCCTCTTTTTGCCCACCAGTAATCCTGATGGTGATATTAATA TCTCCATTCCAGACACAGTAGAAGCACACAGGCAGAACAGTAAAAGGCAGCATCAAGAGAGGGATGGCTACCAGGAGGAAAT CCAGTTGTTAAATAAAGCTTACAGAAAAAGAGAGGAAGAAAGCAAGGGTAGTACAGAAACCAGCCAAGTGGCGCCTGCCTAA
Opto-GPR149	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCCTGTGCGGCGGCGGCGGGCCCTGGGCGGGCCCCAAGCGTCGCAAGCTCAACT ACATCCTGCTCAACCTGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGG GTACTTCGTCTTTGGGCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTG GTGGTCCTGGCCATCGAGCGGTACGTGGTGGTGCGGCTCCCGCACGGCCGGCCGCTGCCCGCCATCATGGGCGTCGCCTTCA CCTGGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTG CGGGATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATC CCCCTGATTGTCATATTCTTCTGCTACGGACACCTACTCTCCGTCTGGTGGCGGCACCGGCCGCGAGGCCCCCGCGGCTGCAG CGCCCTGGTCGGCGAGCCCAGGTCGAGCCCCTGCGCCCAGCGCGCTGCCCCGCGCCAAGGTGCAGAGCCGCATGGTGATCAT CATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGGCGTTCTACATCTTCACCCATCAGGGCTCTGACTTT GGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGCCGTCTACAACCCCGTCATCTACATCATGATGAACA AGCAGTTCCGCCGACAGCTGCGGAAGCGGCTGGGCTCTCTGTGCTGCGCGCCGCAGGGAGGCGCGGAGGACGAGGAGGGGCC CCGGGGCCACCAGGCGCTCTACCGCCAACGCTGGCCCCACCCTCATTATCACCATGCTCGGCGGGAACCGCTGGACGAGGGC GGCTTGCGCCCACCCCCTCCGCGCCCCAGACCCCTGCCTTGCTCCTGCGAAAGTGCCTTCACAGAAACCAGCCAAGTGGCGC CTGCCTAA
Opto-GPR150	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCCTCCTTCACAATGCTTGGAAAGGAAAGCCATCCATGCTCAACTACATCCTGC TCAACCTGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGT CTTTGGGCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTG GCCATCGAGCGGTACGTGGTGGTGAGTGACCCAGCCAAGCAAGTGAGTATCCACAACTACGCCATCATGGGCGTCGCCTTCA CCTGGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTG CGGGATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATC CCCCTGATTGTCATATTCTTCTGCTACGGAAGAGCTTATGACCAATGTAAAAAACGAGGAACTAAGACTCAAAATCTTAGAA ACCAGATACGCTCAAAGCAAGTCACACGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGG

	GGTGGCGTTCTACATCTTCACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACT TCTGCCGTCTACAACCCCGTCATCTACATCATGATGAACAAGCAGTTCCGCGAAGGCTTGAAAGGTGTATGGAAATGGATGA TAACCAAAAAACCTCCAACTGTCTCAGAGTCTCAGGAAACACCAGCTGGCAACTCAGAGGGCCTCCCTGACAAGGTTCCATC TCCAGAATCCCCAGCATCCATACCAGAAAAAGAGAAACCCAGCTCTCCCTCCTCTGGCAAAGGGAAAACTGAGAAGGCAGAG ATTCCCATCCTTCCTGACGTAGAGCAGTTTTGGCATGAGAGGGACACAGTCCCTTCTGTACAGGACAATGACCCTATCCCCT GGGAACATGAAGATCAAGAGACAGGGGAAGGTGTTAAAACAGAAACCAGCCAAGTGGCGCCTGCCTAA
Opto-GPR152	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCGCCGGCTCCCAGGCCCGGCATGGAGCTGGCACGCGACTCAACTACATCCTGC TCAACCTGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGT CTTTGGGCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTG GCCATCGAGCGGTACGTGGTGGTGCTGTGCCCACACTGGTACCCTGGGCACCGCCCAGTCCGCCTGGCCATCATGGGCGTCG CCTTCACCTGGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTG CTCGTGCGGGATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTC ATCATCCCCCTGATTGTCATATTCTTCTGCTACGGACTCACCCAGGCCACAGCCTGTCGCACCTGCCACCGCCAACAGCAGC CCGCAGCCTGCCGGGGCTTCGCCCGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGT GGCGTTCTACATCTTCACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACTTCT GCCGTCTACAACCCCGTCATCTACATCATGATGAACAAGCAGTTCCGCACCCTGCTGCGCTCCGTGCTCTCGTCCTTCGCGG CAGCTCTCTGCGAGGAGCGGCCGGGCAGCTTCACGCCCACTGAGCCACAGACCCAGCTAGATTCTGAGGGTCCAACTCTGCC AGAGCCGATGGCAGAGGCCCAGTCACAGATGGATCCTGTGGCCCAGCCTCAGGTGAACCCCACACTCCAGCCACGATCGGAT CCCACAGCACAGCCACAGCTGAACCCTACGGCCCAGCCACAGTCGGATCCCACAGCCCAGCCACAGCTGAACCTCATGGCCC AGCCACAGTCAGATTCTGTGGCCCAGCCACAGGCAGACACTAACGTCCAGACCCCTGCACCTGCTGCCAGTTCTGTGCCCAG TCCCTGTGATGAAGCTTCCCCAACCCCATCCTCGCATCCTACCCCAGGGGCCCTTGAGGACCCAGCCACACCTCCTGCCTCT GAAGGAGAAAGCCCCAGCAGCACCCCGCCAGAGGCGGCCCCGGGCGCAGGCCCCACGACAGAAACCAGCCAAGTGGCGCCTG CCTAA
Opto-GPR153	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCGCCGGCTCCCAGGCCCGGCATGGAGCTGGCACGCGACTCAACTACATCCTGC TGCAGGAGGACAAGATGCAATACCTGCAGGTCCCGCCCACGCGGCGCTTCTCCCACGACGATGCGGACGTGTGGGCCGCCGT CCCGCTGCCCGCCTTCCTGCCGCGCTGGGGCTCCGGCGAGGACCTGGCCGCCCTGGCGCACCTGGTGCTGCCTGCCGGGCCC GAGCGGCGCCGCGCCAGCCTCCTGGCCTTCGCGGAGGACGCACCACCGTCCCGCGCGCGCCGCCGCTCGGCCGAGAGCCTGC TGTCGCTGCGGCCCTCGGCCCTGGATAGCGGCCCGCGGGGAGCCCGCGACTCGCCCCCCGGCAGCCCGCGCCGCCGCCCGG GCCCGGCCCCGCTCCGCCTCGGCCTCGCTGCTGCCCGACGCCTTCGCCCTGACCGCCTTCGAGTGCGAGCCACAGGCCCTG CGCCGCCCGCCCGGGCCCTTCCCCGCTGCGCCCGCCGCCCCCGACGGCGCAGATCCCGGAGAGGCCCCGACGCCCCCAAGCA GCGCCCAGCGGAGCCCAGGGCCACGCCCCTCTGCGCACTCGCACGCCGGCTCTCTGCGCCCCGGCCTGAGCGCGTCGTGGGG CGAGCCCGGGGGGCTGCGCGCGGCGGGCGGCGGCGGCAGCACCAGCAGCTTCCTGAGTTCCCCCTCCGAGTCCTCGGGCTAC GCCACGCTGCACTCGGACTCGCTGGGCTCCGCGTCCACAGAAACCAGCCAAGTGGCGCCTGCCTAA
Opto-GPR160	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCGCCGGCTCCCAGGCCCGGCATGGAGCTGGCACGCGACTCAACTACATCCTGC TCAACCTGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGT CTTTGGGCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTG GCCATCGAGCGGTACGTGGTGGTGTCTAAAACAACCAAGCTTTCATTTAAGTGTCAAAAATTATTTTATTTCGCCATCATGG GCGTCGCCTTCACCTGGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCAT GCAGTGCTCGTGCGGGATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTC CACTTCATCATCCCCCTGATTGTCATATTCTTCTGCTACGGAAGGATAACTTCCTATATGAATGAAACTATCTTATATTTTC CTTTTTCATCCCACTCCAGTTATACTGTGAGATCTAAAAAACGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTG GCTGCCCTACGCTGGGGTGGCGTTCTACATCTTCACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCT TTCTTTGCCAAGACTTCTGCCGTCTACAACCCCGTCATCTACATCATGATGAACAAGCAGTTCCGCTTAAAAGACATTGGAT TACCTTTGGATCCATTTGTCAACTGGAAGTGCTGCTTCATTCCACTTACAATTCCTAATCTTGAGCAAATTGAAAAGCCTAT ATCAATAATGATTTGTACAGAAACCAGCCAAGTGGCGCCTGCCTAA
Opto-GPR161	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCTTGTACAAGAAGTCCTACCTCCTCACCCTCCTCAACTACATCCTGCTCAACC TGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGG GCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATC GAGCGGTACGTGGTGGTGCTGTACCCCATGGTGTACCCCATGAAGATCACAGGGAACCGGGCCATCATGGGCGTCGCCTTCA CCTGGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTG CGGGATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATC CCCCTGATTGTCATATTCTTCTGCTACGGATTCATCTTCCGCGTGGCCAGGGTCAAGGCACGCAAGGTGCACTGTGGCACAG TCGTCATCGTGGAGGAGGATGCTCAGAGGACCGGGAGGAAGAACTCCAGCACCTCCACCTCCTCTTCAGGCAGCAGGAGGAA TGCCTTTCAGGGTGTGGTCTACTCGGCCAACCAGTGCAAAGCCCTCCGCATGGTGATCATCATGGTCATCGCTTTCCTAATC TGCTGGCTGCCCTACGCTGGGGTGGCGTTCTACATCTTCACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCC CGGCTTTCTTTGCCAAGACTTCTGCCGTCTACAAACCCCGTCATCTACATCATGATGAACAAGCAGTTCCGCAAAGAACTACT GGGCATGTGCTTTGGGGACCGATATTATCGGGAACCATTTGTGCAACGACAGAGGACTTCCAGGCTGTTCAGCATTTCCAAC AGGATCACAGACCTGGGCCTGTCCCCACACCTCACTGCGCTCATGGCAGGTGGACAGCCCCTGGGGCACAGCAGCAGCACGG GGGACACTGGCTTCAGCTGCTCCCAGGACTCAGGGACAGATATGATGCTGCTTGAGGACTACACGTCTGATGACAACCCTCC CTCTCACTGCACTTGCCCACCCAAGAGAAGGAGCTCGGTGACATTTGAGGATGAAGTGGAACAAATCAAAGAAGCTGCCAAG AACTCGATTCTTCATGTGAAAGCTGAAGTACACAAGTCCTTGGACAGTTACGCAGCAAGCTTGGCCAAAGCCATTGAGGCCG

	AAGCCAAAATCAACTTATTTGGGGAGGAGGCTTTGCCAGGGGTCTTGGTTACAGCACGGACTGTCCCGGGGGGCGGCTTCGG GGGCCGCCGAGGCAGCAGAACTCTTGTGAGCCAGAGGCTGCAGTTGCAGAGCATCGAAGAAGGAGATGTTTTAGCTGCCGAG CAGAGAACAGAAACCAGCCAAGTGGCGCCTGCCTAA
	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCATCTCGGCCAAGCAGCAGAAGCACAAGCCACTCAACTACATCCTGCTCAACC TGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGG GCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATC GAGCGGTACGTGGTGGTGCGCTGGCCCGTCAACTACCGCCTCAGCAACGCCAAGAAGCAGGCCATCATGGGCGTCGCCTTCA CCTGGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTG CGGGATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATC CCCCTGATTGTCATATTCTTCTGCTACGGAACACTGTGGGCCCGGCCCCGGAGGGCTCGGCAGGCCCGGAGAGTGGGGGGTG GTGGGGGGACCAAAGCGGGTGGGCCAGGGGCCTTGGGTACCCGGCCAGCTTTTGAGGTACCAGCCATTGTGGTGGAGGATGC CCGAGGGAAGCGGCGGTCCTCGCTGGATGGCTCGGAGTCTGCCAAGACATCCCTGCAGCGCATGGTGATCATCATGGTCATC GCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGGCGTTCTACATCTTCACCCATCAGGGCTCTGACTTTGGCCCCATCT TCATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGCCGTCTACAACCCCGTCATCTACATCATGATGAACAAGCAGTTCCG CGCCGACGTGCGCACAGTGTGGGAGCAATGCGTGGCCATCATGTCTGAGGAGGATGGAGATGACGATGGGGGCTGTGACGAC TATGCAGAGGGCCGAGTTTGCAAAGTTCGCTTTGATGCTAACGGAGCCACAGGACCAGGGAGCCGGGACCCCGCCCAGGTGA AGCTGCTGCCTGGAAGGCACATGCTGTTCCCTCCTCTTGAGAGAGTCCACTACTTACAGGTCCCCCTATCCCGGCGTCTGTC CCATGATGAGACAAACATCTTCTCTACCCCTCGGGAACCAGGCTCCTTCCTGCACAAGTGGTCATCCTCTGATGACATCCGG GTCCTCCCAGCCCAGAGCCGGGCCCTCGGGGGTCCTCCTGAGTACCTGGGACAAAGACACAGGTTGGAGGACGAGGAGGACG AGGAAGAGGCTGAAGGTGGGGGGCTGGCCAGCCTTCGCCAATTCTTGGAGAGTGGGGTTCTGGGGTCAGGTGGGGGACCCCC ACGGGGTCCTGGCTTCTTCCGGGAGGAGATCACCACCTTCATCGATGAGACACCTCTGCCTTCTCCGACTGCCTCACCAGGG CACTCTCCTCGTCGGCCCCGGCCACTGGGCCTCTCACCCCGCCGACTCTCCCTTGGGTCCCCTGAGAGCAGAGCCGTTGGAC TTCCTTTGGGACTAAGCGCAGGGAGGCGCTGCTCCCTGACGGGGGGTGAAGAAAGTGCAAGGGCTTGGGGAGGATCCTGGGG
Opto-GPR162	CCCAGGCAACCCCATCTTTCCCCAGCTGACCCTGACAGAAACCAGCCAAGTGGCGCCTGCCTAA
	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCTTTATACAGAAGAATACGAATCACAGGTGTCTCAACTACATCCTGCTCAACC TGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGG GCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATC GAGCGGTACGTGGTGGTGACACACAGCTGCAAGATCTACCGAATACAAGAACCCGGATTTGCCATCATGGGCGTCGCCTTCA CCTGGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTG CGGGATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATC CCCCTGATTGTCATATTCTTCTGCTACGGACTTGTAATTCGACAGCTCTACAGAAACAAAGATAATGAAAATTACCCAAATG TGAAAAAGGCTCTCCGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGGCGTTCTA CATCTTCACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGCCGTCTAC AACCCCGTCATCTACATCATGATGAACAAGCAGTTCCGCTCAAAGGTCACTGAGACTTTTGCCTCACCTAAAGAAACCAAGG
Opto-GPR171	CTCAGAAAGAAAAATTAAGATGTGAAAATAATGCAACAGAAACCAGCCAAGTGGCGCCTGCCTAA
	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCGTGCTCAAGGAGCGTGCCCTGCACAAGGCTCTCAACTACATCCTGCTCAACC TGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGG GCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATC GAGCGGTACGTGGTGGTGGCCCACCACCGCTTCTACGCCAAGCGCATGACACTCTGGACAGCCATCATGGGCGTCGCCTTCA CCTGGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTG CGGGATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATC CCCCTGATTGTCATATTCTTCTGCTACGGATACCGTCACCGCAAGATGAAGCCAGTGCAGATGGTGCCAGCCATCAGCCAGA ACTGGACATTCCATGGTCCCGGGGCCACCGGCCAGGCTGCTGCCAACTGGATCGCCGGCTTTGGCCGTGGGCCCATGCCACC AACCCTGCTGGGTATCCGGCAGAATGGGCATGCAGCCAGCCGGCGGCTACTGGGCATGGACGAGGTCAAGGGTGAAAAGCAG CTGGGCCGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGGCGTTCTACATCTTCA CCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGCCGTCTACAACCCCGT
Opto-GPR173	CATCTACATCATGATGAACAAGCAGTTCCGCAAGTGCCTGAGGACTCACGCCCCCTGCTGGGGCACAGGAGGTGCCCCGGCT CCCAGAGAACCCTACTGTGTCATGACAGAAACCAGCCAAGTGGCGCCTGCCTAA
	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCTTTTATGGTTATATGAAAGAAACAAAACGACTCAACTACATCCTGCTCAACC TGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGG GCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATC GAGCGGTACGTGGTGGTGATGTACCCCTTTCGCTTCCATGACTGCAAACAGAAATATGACGCCATCATGGGCGTCGCCTTCA CCTGGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTG CGGGATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATC CCCCTGATTGTCATATTCTTCTGCTACGGAAAAACGGTTTTATCACTGCAAGATAAATATCCCATGGCCCAAGATCTTGGAG AGAAACAGAAAGCCTTGCGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGGCGTT CTACATCTTCACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGCCGTC TACAACCCCGTCATCTACATCATGATGAACAAGCAGTTCCGCAGGCGGCTTTCAAGACAAGATTTGCATGACAGCATCCAAC TCCATGCAAAATCCTTTGTGAGTAACCATACAGCTTCCACCATGACACCTGAATTATGCACAGAAACCAGCCAAGTGGCGCC
Opto-GPR174	TGCCTAA
	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCACTTGCCGCACAACCGTGTTCAAATCTGTCCTCAACTACATCCTGCTCAACC TGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGG GCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATC GAGCGGTACGTGGTGGTGCTCTATCCACTGGAGAGGAAAATATCTGATGCCAAGGCCATCATGGGCGTCGCCTTCACCTGGG TCATGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTGCGGGAT TGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATCCCCCTG ATTGTCATATTCTTCTGCTACGGACTGATCCGACGGGCCCTGAGTGCCAGCCAGAAGAAGAAGGTCATCATAGCAGCGCTCC GGACCCCACAGAACACCATCTCTATTCCCTATGCCTCCCAGCGGGAGGCCGAGCTGCACCGCATGGTGATCATCATGGTCAT CGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGGCGTTCTACATCTTCACCCATCAGGGCTCTGACTTTGGCCCCATC
Opto-GPR176	TTCATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGCCGTCTACAACCCCGTCATCTACATCATGATGAACAAGCAGTTCC

	GCAAGTGCTTGATAGGGACCCTGGTGCAACTACACCACCGATACAGTCGCCGTAATGTGGTCAGTACAGGGAGTGGCATGGC TGAGGCCAGCCTGGAACCCAGCATACGCTCGGGTAGCCAGCTCCTGGAGATGTTCCACATTGGGCAGCAGCAGATCTTTAAG CCCACAGAGGATGAGGAAGAGAGTGAGGCCAAGTACATTGGTTCAGCTGACTTCCAGGCCAAGGAGATATTTAGCACCTGCC TGGAGGGAGAGCAGGGGCCACAGTTTGCGCCCTCTGCCCCACCCCTGAGCACAGTGGACTCTGTATCCCAGGTGGCACCGGC AGCCCCTGTGGAACCTGAAACATTCCCTGATAAGTATTCCCTGCAGTTTGGCTTTGGGCCTTTTGAGTTGCCTCCTCAGTGG CTCTCAGAAACCCGAAACAGCAAGAAGCGGCTGCTTCCCCCCTTGGGCAACACCCCAGAGGAGCTGATCCAGACAAAGGTGC CCAAGGTAGGCAGGGTGGAGCGGAAGATGAGCAGAAACAATAAAGTGAGCATTTTTCCAAAGGTGGATTCCACAGAAACCAG CCAAGTGGCGCCTGCCTAA
Opto-GPR182	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCAACTGGCGCGGCTCAGGCCGGGCAGGGCTGCTCAACTACATCCTGCTCAACC TGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGG GCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATC GAGCGGTACGTGGTGGTGACCAGCGCCTCCCCCTCCTGGCAGCGTTACCAGCACCGAGTGCGGCGGGCCGCCATCATGGGCG TCGCCTTCACCTGGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCA GTGCTCGTGCGGGATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCAC TTCATCATCCCCCTGATTGTCATATTCTTCTGCTACGGACTGACAGCCTGCCGGCTGCGGCAGCCAGGACAACCCAAGAGCC GGCGCCACTGCCGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGGCGTTCTACAT CTTCACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGCCGTCTACAAC CCCGTCATCTACATCATGATGAACAAGCAGTTCCGCGGCCGGCTCCTGAATGCTGTAGTCCATTACCTTCCTAAGGACCAGA CCAAGGCGGGCACATGCGCCTCCTCTTCCTCCTGTTCCACCCAGCATTCCATCATCATCACCAAGGGTGATAGCCAGCCTGC TGCAGCAGCCCCCCACCCTGAGCCAAGCCTGAGCTTTCAGGCACACCATTTGCTTCCAAATACTTCCCCCATCTCTCCCACT CAGCCTCTTACACCCAGCACAGAAACCAGCCAAGTGGCGCCTGCCTAA
Opto-GPR183	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAA AGGATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTT CGAGGCCCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGC TTCCCCATCAACTTCCTCACGCTGTACGTCATTGTTCAAAACAGGAAAAAAATCAACTCTCTCAACTACATCCTGCTCAACC TGGCCGTGGCCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGTACTTCGTCTTTGG GCCCACGGGCTGCAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATC GAGCGGTACGTGGTGGTGGTGCACCCTCTACGCTACAACAAGATAAAAAGGATTGAACATGCCATCATGGGCGTCGCCTTCA CCTGGGTCATGGCTCTGGCCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTG CGGGATTGACTACTACACGCCCCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATC CCCCTGATTGTCATATTCTTCTGCTACGGACAGATCTGCTGCAAACTCTTCAGAACTGCCAAACAAAACCCACTCACTGAGA AATCTGGTGTAAACAAAAAGGCTCTCCGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGG GGTGGCGTTCTACATCTTCACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACT TCTGCCGTCTACAACCCCGTCATCTACATCATGATGAACAAGCAGTTCCGCAGAAAGGTTATGAGGATGCTGAAACGGCAAG TCAGTGTATCGATTTCTAGTGCTGTGAAGTCAGCCCCTGAAGAAAATTCACGTGAAATGACAGAAACGCAGATGATGATACA TTCCAAGTCCTCAAATGGAAAGACAGAAACCAGCCAAGTGGCGCCTGCCTAA

Table 6.2 Nucleotide sequences of all light-activated human Class A oGPCRs created using the chimeric algorithm described in section 3.1

Name	**Sequence**
Opto-B2AR	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLGFPI NFLTLYVIAKFERLQTVLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAIERYVVV TSPFKYQSLLTKNKAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFIIPLIVIFFCY GRVFQVAKRQLQKIDKSEGRFHSPNLGQVEQDGRSGHGLRRSSKFCLKEHKALRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDF GPIFMTIPAFFAKTSAVYNPVIYIMMNKQFRIAFQELLCLRRSSSKAYGNGYSSNSNGKTDYMGEASGCQLGQEKESERLCEDPP GTESFVNCQGTVPSLSLDSQGRNCSTNDSPLTETSQVAPA*
Opto-A1AR	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLGFPI NFLTLYVVACHRHLHSVLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAIERYVVV SYPLRYPTIVTQRRAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFIIPLIVIFFCY GRVYVVAKRESRGLKSGLKTDKSDSEQVTLRIHRKNAPAGGSGMASAKTKTHFSVRLLKFSREKKAARMVIIMVIAFLICWLPYA GVAFYIFTHQGSDFGPIFMTIPAFFAKTSAVYNPVIYIMMNKQFRKAFQNVLRIQCLCRKQSSKHALGYTLHPPSQAVEGQHKDM VRIPVGSRETFYRISKTDGVCEWKFFSSMPRGSARITVSKDQSSCTTARVRSKSFLQVCCCVGPSTPSLDKNHQVPTIKVHTISL SENGEEVTETSQVAPA*
Opto-FFR3	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLGFPI NFLTLYVFVGKLQRRPVALNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAIERYVV VAHPLWYKTRPRLGQAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFIIPLIVIFFC YGRLVWILGRGGSHRRQRRVARMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSAVYNPVIYIMMNKQFRA DFHELLRRLCGLWGQWQQESSMELKEQKGGEEQRADRPAERKTSEHSQGCGTGGQVACAESTETSQVAPA*
Opto-A2A	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLGFPI NFLTLYVVWLNSNLQNVLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAIERYVVV RIPLRYNGLVTGTRAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFIIPLIVIFFCY GRIFLAARRQLKQMESQPLPGERARSTLQKEVHARMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSAVYN PVIYIMMNKQFRQTFRKIIRSHVLRQQEPFKAAGTSARVLAAHGSDGEQVSLRLNGHPPGVWANGSAPHPERRPNGYALGLVSGG SAQESQGNTGLPDVELLSHELKGVCPEPPGLDDPLAQDGAGVSTETSQVAPA*
Opto-D1R	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLGFPI NFLTLYVVIRFRHLRSKVLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAIERYVV VSSPFQYERKMTPKAAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFIIPLIVIFFC YGSIYRIAQKQIRRISALERAAVHAKNCQTTTGNGNPVECSQSESSFKMSFKRETKVLRMVIIMVIAFLICWLPYAGVAFYIFTH QGSDFGPIFMTIPAFFAKTSAVYNPVIYIMMNKQFRKAFSTLLGCYRLCPTTNNAIETVSINNNGAVMFSSHHEPRGSISKDCNL VYLIPHAVGSSEDLKREEAGGIPKPLEKLSPALSVILDYDTDVSLEKIQPVTHSGQHSTTETSQVAPA*
Opto-D2R	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLGFPI NFLTLYVVSREKALQTTLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAIERYVVV AMPMLYNTRYSSKRRAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFIIPLIVIFFC YGKIYIVLRKRRKRVNTKRSSRAFRANLKTPLKGNCTHPEDMKLCTVIMKSNGSFPVNRRRMDAARRAQELEMEMLSSTSPPERT RYSPIPPSHHQLTLPDPSHHGLHSNPDSPAKPEKNGHAKIVNPRIAKFFEIQTMPNGKTRTSLKTMSRRKLSQQKEKKATRMVII MVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSAVYNPVIYIMMNKQFRKAFMKILHCTETSQVAPA*

Opto-M1R	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLGFPI NFLTLYVFKVNTELKTVLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAIERYVVV TRPLSYRAKRTPRRAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFIIPLIVIFFCY GRIYRETENRARELAALQGSETPGKGGGSSSSSERSQPGAEGSPETPPGRCCRCCRAPRLLQAYSWKEEEEEDEGSMESLTSSEG EEPGSEVVIKMPMVDPEAQAPTKQPPRSSPNTVKRPTKKGRDRAGKGQKPRGKEQLAKRKTFSLVKEKKAARMVIIMVIAFLICW LPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSAVYNPVIYIMMNKQFRDTFRLLLLCRWDKRRWRKIPKRPGSVHRTPSRQCTE TSQVAPA*
Opto-M2R	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLGFPI NFLTLYVIKVNRHLQTVLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAIERYVVV TKPLTYPVKRTTKMAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFIIPLIVIFFCY GHISRASKSRIKKDKKEPVANQDPVSPSLVQGRIVKPNNNNMPSSDDGLEHNKIQNGKAPRDPVTENCVQGEEKESSNDSTSVSA VASNMRDDEITQDENTVSTSLGHSKDENSKQTCIRIGTKTPKSDSCTPTNTTVEVVGSSGQNGDEKQNIVARKIVKMTKQPAKKK PPPSREKKVTRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSAVYNPVIYIMMNKQFRKTFKHLLMCHYK NIGATRTETSQVAPA*
Opto-M3R	MKTIIALSYIFCLVFAMYTDIEMNRLGKDSLMNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLGFPI NFLTLYVFKVNKQLKTVLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAIERYVVV TRPLTYRAKRTTKRAIMGVAFTWVMALACAAPPLVGWSRYIPEGMQCSCGIDYYTPHEETNNESFVIYMFVVHFIIPLIVIFFCY GRIYKETEKRTKELAGLQASGTEAETENFVHPTGSSRSCSSYELQQQSMKRSNRRKYGRCHFWFTTKSWKPSSEQMDQDHSSSDS WNNNDAAASLENSASSDEEDIGSETRAIYSIVLKLPGHSTILNSTKLPSSDNLQVPEEELGMVDLERKADKLQAQKSVDDGGSFP KSFSKLPIQLESAVDTAKTSDVNSSVGKSTATLPLSFKEATLAKRFALKTRSQITKRKRMSLVKEKKAARMVIIMVIAFLICWLP YAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSAVYNPVIYIMMNKQFRTTFKMLLLCQCDKKKRRKQQYQQRQSVIFHKRAPEQAL TETSQVAPA*

Table 6.3 Amino acid sequences of all light-activated control GPCRs created using the chimeric algorithm described in section 3.1

Name	**Sequence**
Opto-B2AR	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAAAGG ATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTTCGAGGC CCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGCTTCCCCATC AACTTCCTCACGCTGTACGTCATTGCCAAGTTCGAGAGGCTACAGACTGTCCTCAACTACATCCTGCTCAACCTGGCCGTGGCCG ACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGGGCCCACGGGCTGCAA CCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATCGAGCGGTACGTGGTGGTG ACATCGCCATTCAAGTACCAGAGCCTGCTGACCAAGAATAAGGCCATCATGGGCGTCGCCTTCACCTGGGTCATGGCTCTGGCCT GTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTGCGGGATTGACTACTACACGCCCCA CGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATCCCCCTGATTGTCATATTCTTCTGCTAC GGAAGGGTGTTCCAGGTGGCCAAAAGGCAGCTCCAGAAGATAGACAAATCTGAGGGAAGATTCCACTCCCCAAACCTCGGCCAGG TGGAGCAGGATGGGCGGAGTGGGCACGGACTCCGAAGGTCCTCCAAGTTCTGCTTGAAGGAGCACAAAGCCCTCCGCATGGTGAT CATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGGCGTTCTACATCTTCACCCATCAGGGCTCTGACTTT GGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGCCGTCTACAACCCCGTCATCTACATCATGATGAACAAGC AGTTCCGCATTGCCTTCCAGGAGCTTCTATGCCTCCGCAGGTCCTCTTCAAAAGCCTATGGGAACGGCTACTCCAGCAACAGTAA TGGCAAAACAGACTACATGGGGGAGGCGAGTGGATGTCAGCTGGGGCAGGAAAAAGAAAGTGAACGGCTGTGTGAGGACCCCCCA GGCACGGAAAGCTTTGTGAACTGTCAAGGTACTGTGCCTAGCCTTAGCCTTGATTCCCAAGGGAGGAACTGTAGTACAAATGACT CACCGCTGACAGAAACCAGCCAAGTGGCGCCTGCCTAA
Opto-A1AR	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAAAGG ATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTTCGAGGC CCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGCTTCCCCATC AACTTCCTCACGCTGTACGTCGTAGCCTGTCACCGACACCTGCACTCAGTCCTCAACTACATCCTGCTCAACCTGGCCGTGGCCG ACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGGGCCCACGGGCTGCAA CCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATCGAGCGGTACGTGGTGGTG AGCTACCCGCTGCGCTACCCAACCATCGTCACCCAGAGGAGGGCCATCATGGGCGTCGCCTTCACCTGGGTCATGGCTCTGGCCT GTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTGCGGGATTGACTACTACACGCCCCA CGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATCCCCCTGATTGTCATATTCTTCTGCTAC GGACGCGTCTACGTGGTGGCCAAGAGGGAGAGCCGGGGCCTCAAGTCTGGCCTCAAGACCGACAAGTCGGACTCGGAGCAAGTGA CGCTCCGCATCCATCGGAAAAACGCCCCGGCAGGAGGCAGCGGGATGGCCAGCGCCAAGACCAAGACGCACTTCTCAGTGAGGCT CCTCAAGTTCTCCCGGGAGAAGAAAGCGGCCCGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCT GGGGTGGCGTTCTACATCTTCACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACTT CTGCCGTCTACAACCCCGTCATCTACATCATGATGAACAAGCAGTTCCGCAAGGCCTTTCAGAATGTCTTGAGAATCCAGTGTCT CTGCAGAAAGCAGTCCTCCAAACATGCCCTGGGCTACACCCTGCACCCGCCCAGCCAGGCCGTGGAAGGGCAACACAAGGACATG GTGCGCATCCCCGTGGGATCAAGAGAAACCTTCTACAGGATCTCCAAGACGGATGGCGTTTGTGAATGGAAATTTTTCTCTTCCA TGCCCCGTGGATCTGCCAGGATTACAGTGTCCAAAGACCAATCCTCCTGTACCACAGCCCGGGTGAGAAGTAAAAGCTTTTTGCA GGTCTGCTGCTGTGTAGGGCCCTCAACCCCCAGCCTTGACAAGAACCATCAAGTTCCAACCATTAAGGTCCACACCATCTCCCTC AGTGAGAACGGGGAGGAAGTCACAGAAACCAGCCAAGTGGCGCCTGCCTAA
Opto-FFR3	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAAAGG ATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTTCGAGGC CCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGCTTCCCCATC AACTTCCTCACGCTGTACGTCTTTGTGGGCAAGCTGCAGCGCCGCCGGTGGCCCTCAACTACATCCTGCTCAACCTGGCCGTGG CCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGGGCCCACGGGCTG CAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATCGAGCGGTACGTGGTG GTGGCCCACCCACTGTGGTACAAGACCCGGCCGAGGCTGGGGCAGGCCATCATGGGCGTCGCCTTCACCTGGGTCATGGCTCTGG CCTGTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTGCGGGATTGACTACTACACGCC CCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATCCCCCTGATTGTCATATTCTTCTGC TACGGACGCCTGGTGTGGATCCTCGGCAGAGGGGGCAGCCACCGCCGGCAGAGGAGGGTGGCGCGCATGGTGATCATCATGGTCA TCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGGCGTTCTACATCTTCACCCATCAGGGCTCTGACTTTGGCCCCATCTT CATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGCCGTCTACAACCCCGTCATCTACATCATGATGAACAAGCAGTTCCGCGCC GACTTTCATGAGCTGCTGAGGAGGTTGTGTGGGCTCTGGGGCCAGTGGCAGCAGGAGAGCAGCATGGAGCTGAAGGAGCAGAAGG GAGGGGAGGAGCAGAGAGCGGACCGACCAGCTGAAAGAAAGACCAGTGAACACTCACAGGGCTGTGGAACTGGTGGCCAGGTGGC CTGTGCTGAAAGCACAGAAACCAGCCAAGTGGCGCCTGCCTAA
Opto-A2A	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAAAGG ATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTTCGAGGC

	CCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGCTTCCCCATC AACTTCCTCACGCTGTACGTCGTGTGGCTCAACAGCAACCTGCAGAACGTCCTCAACTACATCCTGCTCAACCTGGCCGTGGCCG ACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGGGCCCACGGGCTGCAA CCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATCGAGCGGTACGTGGTGGTG CGCATCCCGCTCCGGTACAATGGCTTGGTGACCGGCACGAGGGCCATCATGGGCGTCGCCTTCACCTGGGTCATGGCCTCTGGCCT GTGCCGCGCCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTGCGGGATTGACTACTACACGCCCCA CGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATCCCCCTGATTGTCATATTCTTCTGCTAC GGACGGATCTTCCTGGCGGCGCGACGACAGCTGAAGCAGATGGAGAGCCAGCCTCTGCCGGGGGAGCGGGCACGGTCCACACTGC AGAAGGAGGTCCATGCTCGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGGCGTTCTA CATCTTCACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGCCGTCTACAAC CCCGTCATCTACATCATGATGAACAAGCAGTTCCGCCAGACCTTCCGCAAGATCATTCGCAGCCACGTCCTGAGGCAGCAAGAAC CTTTCAAGGCAGCTGGCACCAGTGCCCGGGTCTTGGCAGCTCATGGCAGTGACGGAGAGCAGGTCAGCCTCCGGCTCAACGGCCA CCCGCCAGGAGTGTGGGCCAACGGCAGTGCTCCCCACCCTGAGCGGAGGCCCAATGGCTATGCCCTGGGGCTGGTGAGTGGAGGG AGTGCCCAAGAGTCCCAGGGGAACACGGGCCTCCCAGACGTGGAGCTCCTTAGCCATGAGCTCAAGGGAGTGTGCCCAGAGCCCC CTGGCCTAGATGACCCCCTGGCCCAGGATGGAGCAGGAGTGTCCACAGAAACCAGCCAAGTGGCGCCTGCCTAA
Opto-D1R	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAAAGG ATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTTCGAGGC CCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGATCGGCCGCCTACATGTTCCTGCTGATCATGCTTGGCTTCCCCATC AACTTCCTCACGCTGTACGTCGTCATCAGGTTTCGACACCTGCGGTCCAAGGTGCTCAACTACATCCTGCTCAACCTGGCCGTGG CCGACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGGGCCCACGGGCTG CAACCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATCGAGCGGTACGTGGTG GTGTCCAGCCCTTTCCAGTATGAGAGGAAGATGACTCCGAAGGCAGCCATCATGGGCGTCGCCTTCACCTGGGTCATGGCTCTGG CCTGTGCCGCGCCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTGCGGGATTGACTACTACACGCC CCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATCCCCCTGATTGTCATATTCTTCTGC TACGGAAGTATCTACAGGATTGCCCAGAAGCAAATCCGGCGCATCTCAGCTTTGGAGAGGGCAGCAGTCCATGCCAAGAACTGCC AGACCACCACAGGTAATGGAAACCCTGTCGAATGCTCTCAATCAGAAAGTTCCTTTAAGATGTCCTTTAAGAGGGAGACTAAAGT CCTGCGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGGCGTTCTACATCTTCACCCAT CAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGCCGTCTACAACCCCGTCATCTACA TCATGATGAACAAGCAGTTCCGCAAGGCATTCTCGACCCTCTTAGGATGCTATAGACTCTGCCCTACAACGAATAATGCCATAGA GACTGTAAGCATCAACAACAACGGGGCTGTGATGTTTTCCAGCCACCATGAGCCCCGAGGCTCCATCTCCAAGGACTGTAATCTG GTTTACCTGATCCCTCATGCTGTGGGCTCCTCTGAGGACCTGAAGAGGGAGGAGGCCGGTGGCATACCTAAGCCACTGGAGAAGC TGTCCCCGGCCTTATCGGTCATATTGGACTATGACACCGATGTCTCTCTAGAAAAGATCCAACCCGTTACCCACAGCGGACAGCA TTCAACCACAGAAACCAGCCAAGTGGCGCCTGCCTAA
Opto-D2R	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAAAGG ATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTTCGAGGC CCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGCTTCCCCATC AACTTCCTCACGCTGTACGTCGTATCACGAGAGAAGGCTTTGCAGACCACCCTCAACTACATCCTGCTCAACCTGGCCGTGGCCG ACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGGGCCCACGGGCTGCAA CCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATCGAGCGGTACGTGGTGGTG GCCATGCCTATGTTGTATAACACACGCTACAGCTCCAAGCGCCGAGCCATCATGGGCGTCGCCTTCACCTGGGTCATGGCTCTGG CCTGTGCCGCGCCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTGCGGGATTGACTACTACACGCC CCACGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATCCCCCTGATTGTCATATTCTTCTGC TACGGAAAAATCTACATCGTTCTCCGCAAGCGTCGGAAGCGGGTCAACACCAAGCGTAGCAGCCGAGCTTTCAGAGCCAACCTGA AAACACCACTCAAGGGCAACTGTACCCACCCTGAGGACATGAAACTCTGCACCGTTATCATGAAGTCTAATGGGAGTTTCCCAGT GAACAGGCGGAGAATGGATGCTGCCCGCCGAGCTCAGGAGCTGGAAATGGAGATGCTGTCAAGCACCAGCCCCCCAGAGAGGACC CGGTATAGCCCCATCCCTCCCAGTCACCACCAGCTCACTCTCCCCGATCCATCCCACCACGGTCTACATAGCAACCCTGACAGTC CTGCCAAACCAGAAAAGAATGGGCATGCCAAGATTGTCAATCCCAGGATTGCCAAGTTCTTTGAGATCCAGACCATGCCCAATGG CAAAACCCGGACCTCCCTTAAGACGATGAGCCGCAGGAAGCTCTCCCAGCAGAAGGAGAAGAAAGCCACTCGCATGGTGATCATC ATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTGGGGTGGCGTTCTACATCTTCACCCATCAGGGCTCTGACTTTGGCC CCATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACTTCTGCCGTCTACAACCCCGTCATCTACATCATGATGAACAAGCAGTT CCGCAAGGCCTTCATGAAGATCCTGCACTGCACAGAAACCAGCCAAGTGGCGCCTGCCTAA
Opto-M1R	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAAAGG ATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTTCGAGGC CCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGCTTCCCCATC AACTTCCTCACGCTGTACGTCTTCAAGGTCAACACGGAGCTCAAGACAGTCCTCAACTACATCCTGCTCAACCTGGCCGTGGCCG ACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGGGCCCACGGGCTGCAA CCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATCGAGCGGTACGTGGTGGTG ACTCGGCCCCTGAGCTACCGTGCCAAGCGCACACCCCGCCGGGCCATCATGGGCGTCGCCTTCACCTGGGTCATGGCTCTGGCCT GTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTGCGGGATTGACTACTACACGCCCCA CGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATCCCCCTGATTGTCATATTCTTCTGCTAC GGACGCATCTACCGGGAGACAGAGAACCGAGCACGGGAGCTGGCAGCCCTTCAGGGCTCCGAAACGCCAGGCAAAGGGGGTGGCA GCAGCAGCAGCTCAGAGAGGTCCCAGCCAGGGGCTGAGGGCTCACCAGAGACTCCTCCAGGCCGCTGCTGTCGCTGCTGCCGGGC CCCCAGGCTGCTGCAGGCCTACAGCTGGAAGGAAGAAGAGGAAGAGGACGAAGGCTCCATGGAGTCCCTCACATCCTCAGAGGGA GAGGAGCCTGGCTCCGAAGTGGTGATCAAGATGCCAATGGTGGACCCCGAGGCACAGGCCCCCACCAAGCAGCCCCACGGAGCT CCCCAAATACAGTCAAGAGGCCGACTAAGAAAGGGCGTGATCGAGCTGGCAAGGGCCAGAAGCCCCGTGGAAAGGAGCAGCTGGC CAAGCGGAAAACCTTCTCGCTGGTCAAGGAGAAGAAGGCGGCTCGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGG CTGCCCTACGCTGGGGTGGCGTTCTACATCTTCACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCT TTGCCAAGACTTCTGCCGTCTACAACCCCGTCATCTACATCATGATGAACAAGCAGTTCCGCGACACCTTTCGCCTGCTGCTGCT TTGCCGCTGGGACAAGAGGCGCTGGCGCAAGATCCCCAAGCGCCCTGGCTCCGTGCACCGCACTCCCTCCCGCCAATGCACAGAA ACCAGCCAAGTGGCGCCTGCCTAA
Opto-M2R	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAAAGG ATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTTCGAGGC CCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGCTTCCCCATC AACTTCCTCACGCTGTACGTCATTAAAGTCAACCGCCACCTCCAGACCGTCCTCAACTACATCCTGCTCAACCTGGCCGTGGCCG ACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGGGCCCACGGGCTGCAA CCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATCGAGCGGTACGTGGTGGTG ACAAAACCTCTGACCTACCCAGTCAAGCGGACCACAAAAATGGCCATCATGGGCGTCGCCTTCACCTGGGTCATGGCTCTGGCCT GTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTGCGGGATTGACTACTACACGCCCCA CGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATCCCCCTGATTGTCATATTCTTCTGCTAC GGACACATATCCCGAGCCAGCAAGAGCAGGATAAAGAAGGACAAGAAGGAGCCTGTTGCCAACCAAGACCCCGTTTCTCCAAGTC TGGTACAAGGAAGGATAGTGAAGCCAAACAATAACAACATGCCCAGCAGTGACGATGGCCTGGAGCACAACAAAATCCAGAATGG CAAAGCCCCCAGGGATCCTGTGACTGAAAACTGTGTTCAGGGAGAGGAGAAGGAGAGCTCCAATGACTCCACCTCAGTCAGTGCT GTTGCCTCTAATATGAGAGATGATGAAATAACCCAGGATGAAAACACAGTTTCCACTTCCCTGGGCCATTCCAAAGATGAGAACT CTAAGCAAACATGCATCAGAATTGGCACCAAGACCCCAAAAAGTGACTCATGTACCCCAACTAATACCACCGTGGAGGTAGTGGG GTCCTCAGGTCAGAATGGAGATGAAAAGCAGAATATTGTAGCCCGCAAGATTGTGAAGATGACTAAGCAGCCTGCAAAAAAGAAG

	CCTCCTCCTTCCCGGGAAAAGAAAGTCACCCGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCCTACGCTG GGGTGGCGTTCTACATCTTCACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCAAGACTTC TGCCGTCTACAACCCCGTCATCTACATCATGATGAACAAGCAGTTCCGCAAAACCTTTAAACACCTTCTCATGTGTCATTATAAG AACATAGGCGCTACAAGGACAGAAACCAGCCAAGTGGCGCCTGCCTAA
Opto-M3R	ATGAAAACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCATGTACACCGATATAGAGATGAACAGGCTGGGAAAGG ATAGCCTCATGAACGGGACCGAGGGCCCAAACTTCTACGTGCCTTTCTCCAACAAGACGGGCGTGGTGCGCAGCCCCTTCGAGGC CCCGCAGTACTACCTGGCGGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTCCTGCTGATCATGCTTGGCTTCCCCATC AACTTCCTCACGCTGTACGTCTTTAAGGTCAACAAGCAGCTGAAGACGGTCCTCAACTACATCCTGCTCAACCTGGCCGTGGCCG ACCTCTTCATGGTGTTCGGGGGCTTCACCACCACCCTCTACACCTCTCTGCACGGGTACTTCGTCTTTGGGCCCACGGGCTGCAA CCTGGAGGGCTTCTTTGCCACCTTGGGCGGTGAAATTGCACTGTGGTCCTTGGTGGTCCTGGCCATCGAGCGGTACGTGGTGGTG ACGAGGCCGCTCACGTACCGAGCCAAACGAACAACAAAGAGAGCCATCATGGGCGTCGCCTTCACCTGGGTCATGGCTCTGGCCT GTGCCGCGCCCCCCCTCGTCGGCTGGTCCAGGTACATCCCGGAGGGCATGCAGTGCTCGTGCGGGATTGACTACTACACGCCCCA CGAGGAAACCAACAATGAGTCGTTCGTCATCTACATGTTCGTGGTCCACTTCATCATCCCCCTGATTGTCATATTCTTCTGCTAC GGAAGGATCTATAAGGAAACTGAAAAGCGTACCAAAGAGCTTGCTGGCCTGCAAGCCTCTGGGACAGAGGCAGAGACAGAAAACT TTGTCCACCCCACGGGCAGTTCTCGAAGCTGCAGCAGTTACGAACTTCAACAGCAAAGCATGAAACGCTCCAACAGGAGGAAGTA TGGCCGCTGCCACTTCTGGTTCACAACCAAGAGCTGGAAACCCAGCTCCGAGCAGATGGACCAAGACCACAGCAGCAGTGACAGT TGGAACAACAATGATGCTGCTGCCTCCCTGGAGAACTCCGCCTCCTCCGACGAGGAGGACATTGGCTCCGAGACGAGAGCCATCT ACTCCATCGTGCTCAAGCTTCCGGGTCACAGCACCATCCTCAACTCCACCAAGTTACCCTCATCGGACAACCTGCAGGTGCCTGA GGAGGAGCTGGGGATGGTGGACTTGGAGAGGAAAGCCGACAAGCTGCAGGCCCAGAAGAGCGTGGACGATGGAGGCAGTTTTCCA AAAAGCTTCTCCAAGCTTCCCATCCAGCTAGAGTCAGCCGTGGACACAGCTAAGACTTCTGACGTCAACTCCTCAGTGGGTAAGA GCACGGCCACTCTACCTCTGTCCTTCAAGGAAGCCACTCTGGCCAAGAGGTTTGCTCTGAAGACCAGAAGTCAGATCACTAAGCG GAAAAGGATGTCCCTGGTCAAGGAGAAGAAAGCGGCCCGCATGGTGATCATCATGGTCATCGCTTTCCTAATCTGCTGGCTGCCC TACGCTGGGGTGGCGTTCTACATCTTCACCCATCAGGGCTCTGACTTTGGCCCCATCTTCATGACCATCCCGGCTTTCTTTGCCA AGACTTCTGCCGTCTACAACCCCGTCATCTACATCATGATGAACAAGCAGTTCCGCACCACTTTCAAGATGCTGCTGCTGTGCCA GTGTGACAAAAAAAAAGAGGCGCAAGCAGCAGTACCAGCAGAGACAGTCGGTCATTTTTCACAAGCGCGCACCCGAGCAGGCCTTG ACAGAAACCAGCCAAGTGGCGCCTGCCTAA

Table 6.4 Nucleotide sequences of all light-activated control GPCRs created using the chimeric algorithm described in section 3.1

	CRE		SRE		SRE.L		CRE+stim.	
Gene name	**Dark**	**Light**	**Dark**	**Light**	**Dark**	**Light**	**Dark**	**Light**
Opto-GPR1	1.52 ± 0.42	8.94 ± 3 52	0 52 ± 0.17	0.61 ± 0 22	0.74 ± 0.25	0 57 ± 0.20	1.39 ± 0.12	1.45 ± 0.26
Opto-GPR3	0.45 ± 0.16	0.33 ± 0 07	0 68 ± 0.23	3.63 ± 2 95	0 63 ± 0.19	0.44 ± 0.17	1.03 ± 0.13	0 92 ± 0.18
Opto-GPR4	0.47 ± 0.18	0 294 ± 0.07	1 61 ± 1.00	1.84 ± 1 36	0 67 ± 0.25	0 50 ± 0.38	0.60 ± 0.12	0 36 ± 0.11
Opto-GPR6	0.49 ± 0.26	0.25 ± 0 08	1 26 ± 0.84	1.09 ± 0.46	0 69 ± 0.21	0 51 ± 0.20	1.02 ± 0 08	0.83 ± 0 2
Opto-GPR12	0.19 ± 0.08	0.2 ± 0.06	1.17 ± 0.58	0.76 ± 0 30	0.8 ± 0.28	0 58 ± 0.27	1.14 ± 0.19	0 95 ± 0.15
Opto-GPR15	0.26 ± 0.14	0.21 ± 0 07	0 54 ± 0.29	0.65 ± 0 31	0.77 ± 0.25	0.73 ± 0.32	1.02 ± 0.13	0.77 ± 0.13
Opto-GPR17	0.32 ± 0.10	0.27 ± 0 08	0.49 ± 0.25	0.6 ± 0.24	0 87 ± 0.34	0 54 ± 0.17	1 ± 0.08	0 83 ± 0.15
Opto-GPR18	0.19 ± 0.06	0.22 ± 0 06	0 32 ± 0.07	0.8 ± 0.49	0 87 ± 0.28	0 56 ± 0.21	1 ± 0.12	1.14 ± 0.33
Opto-GPR19	0.21 ± 0.08	0.24 ± 0 09	0.46 ± 0.23	0.55 ± 0 25	0.73 ± 0.22	0 63 ± 0.28	1.03 ± 0.14	0.9 ± 0.21
Opto-GPR20	0.23 ± 0.03	0.49 ± 0 20	0.49 ± 0.20	0.86 ± 0 29	0 59 ± 0.15	0 64 ± 0.24	0.88 ± 0.10	0.74 ± 0.19
Opto-GPR21	0.36 ± 0.07	10.36 ± 5.73	1 66 ± 1.36	0.57 ± 0 22	0 62 ± 0.19	0 64 ± 0.22	0.86 ± 0 08	0 99 ± 0.31
Opto-GPR22	0.27 ± 0.09	0.41 ± 0.12	1.41 ± 0.74	1.27 ± 0 59	0 69 ± 0.18	0 86 ± 0.31	1.05 ± 0 29	2.45 ± 1.34
Opto-GPR25	0.29 ± 0.08	0.4 ± 0.08	0 97 ± 0.61	1.44 ± 1 03	0 64 ± 0.22	0.45 ± 0.15	1.29 ± 0.17	0 98 ± 0.19
Opto-GPR26	0.36 ± 0.10	0.41 ± 0 09	1.2 ± 0.84	0.54 ± 0.14	0 53 ± 0.17	0.44 ± 0.15	1.15 ± 0.11	0 86 ± 0.15
Opto-GPR27	0.32 ± 0.10	0.23 ± 0 04	0 56 ± 0.20	0.59 ± 0 21	0 58 ± 0.17	0.41 ± 0.15	1.01 ± 0 06	0 89 ± 0.14
Opto-GPR31	0.27 ± 0.11	0.57 ± 0 27	0 56 ± 0.19	0.79 ± 0 38	0 57 ± 0.21	0 51 ± 0.17	1.02 ± 0.10	0.8 ± 0.15
Opto-GPR32	0.21 ± 0.07	4.89 ± 2 32	0 81 ± 0.38	0.67 ± 0 36	0 54 ± 0.16	0.44 ± 0.13	1.04 ± 0 09	0 66 ± 0.15
Opto-GPR33	0.31 ± 0.14	0.46 ± 0.10	0.3 ± 0.07	2.45 ± 1 33	0 62 ± 0.16	30.56 ± 10.2 3	0.97 ± 0 09	0 65 ± 0.13
Opto-GPR34	0 3 ± 0.11	0.3 ± 0.09	0.49 ± 0.16	0.5 ± 0.14	0 61 ± 0.27	0 65 ± 0.16	0.95 ± 0 09	0 81 ± 0.09
Opto-GPR35	0.32 ± 0.11	0.22 ± 0 04	0 33 ± 0.08	0.47 ± 0.16	0 63 ± 0.16	0 64 ± 0.21	1.03 ± 0.10	0.74 ± 0.12
Opto-GPR37	0 3 ± 0 08	0.25 ± 0 07	0.5 ± 0.11	0.41 ± 0.13	0 53 ± 0.12	0.47 ± 0.16	0.91 ± 0 08	0 91 ± 0.15
Opto-GPR37L1	0.25 ± 0.08	0.31 ± 0 09	0 33 ± 0.11	0.33 ± 0 08	0.49 ± 0.13	0.5 ± 0.19	0.89 ± 0.11	1 06 ± 0.20

Opto-GPR39	0.39 ± 0.19	0.51 ± 0 21	0 67 ± 0.21	0.35 ± 0.10	0 52 ± 0.15	0 63 ± 0.19	0.87 ± 0 05	0.92 ± 0.21
Opto-GPR42	0 3 ± 0.14	1.05 ± 0 68	0 59 ± 0.21	0.49 ± 0.18	0 61 ± 0.15	0.6 ± 0.20	0.88 ± 0 05	1.22 ± 0.46
Opto-GPR45	0.43 ± 0.16	0.56 ± 0.12	1 38 ± 0.60	0.71 ± 0 24	0 53 ± 0.13	0.48 ± 0.15	1.16 ± 0.18	0.85 ± 0.07
Opto-GPR50	0.4 ± 0.16	0.43 ± 0 06	0 81 ± 0.20	0.86 ± 0 28	0 51 ± 0.15	0 34 ± 0.12	1.07 ± 0.19	1.07 ± 0.13
Opto-GPR52	0.26 ± 0.09	0.3 ± 0.07	0 93 ± 0.49	0.65 ± 0 24	0 54 ± 0.15	0.45 ± 0.17	0.99 ± 0 07	0.88 ± 0.11
Opto-GPR55	0.32 ± 0.12	0.46 ± 0 09	0.74 ± 0 2	0.66 ± 0 20	0.5 ± 0.15	0.4 ± 0.10	1.12 ± 0.10	0.75 ± 0.15
Opto-GPR61	0.62 ± 0.17	6.11 ± 3.10	0.58 ± 0 2	0.66 ± 0 29	0 51 ± 0.15	0 62 ± 0.18	1.07 ± 0.12	0.82 ± 0.12
Opto-GPR62	0.32 ± 0.09	0.51 ± 0.14	0 57 ± 0.22	0.77 ± 0 22	0 55 ± 0.16	0.6 ± 0.11	0.89 ± 0 04	0.83 ± 0.15
Opto-GPR63	0.37 ± 0.11	0.51 ± 0.13	0.42 ± 0.12	0.44 ± 0.16	0 53 ± 0.18	0.78 ± 0.16	1.16 ± 0 28	0.74 ± 0.15
Opto-GPR65	0.39 ± 0.15	0.53 ± 0 21	0 59 ± 0.19	0.67 ± 0 26	0 54 ± 0.14	0 58 ± 0.16	1.02 ± 0 05	0 8 ± 0 08
Opto-GPR68	0.81 ± 0.45	0.78 ± 0 31	0 97 ± 0.27	2.61 ± 0.76	0 52 ± 0.15	0 58 ± 0.17	0.84 ± 0 05	1.02 ± 0.09
Opto-GPR75	0.33 ± 0.14	0.30 ± 0 08	0 99 ± 0.45	1.28 ± 0 93	0 61 ± 0.18	0 62 ± 0.15	0.89 ± 0 09	0.86 ± 0.13
Opto-GPR78	0.29 ± 0.05	0.65 ± 0 24	1 ± 0.34	5.05 ± 2 87	0 54 ± 0.12	35.1 ± 11.47	0.96 ± 0.13	1.38 ± 0.32
Opto-GPR82	0.28 ± 0.07	0.50 ± 0.14	0 59 ± 0.19	0.54 ± 0 21	0 68 ± 0.17	0.71 ± 0.15	0.93 ± 0.14	1.31 ± 0.43
Opto-GPR83	0.81 ± 0.18	0.67 ± 0.14	0 95 ± 0.19	0.86 ± 0 25	0 56 ± 0.22	0 51 ± 0.16	1.23 ± 0 26	1.09 ± 0.17
Opto-GPR84	0.47 ± 0.09	1.02 ± 0 27	0.75 ± 0.23	0.67 ± 0.19	0.49 ± 0.16	0.45 ± 0.13	1.03 ± 0.14	1.04 ± 0.12
Opto-GPR85	0.55 ± 0.15	1.10 ± 0 38	0 54 ± 0.16	0.43 ± 0.10	0 39 ± 0.10	0.49 ± 0.15	0.97 ± 0.13	0.72 ± 0.15
Opto-GPR87	0.44 ± 0.09	0.53 ± 0 09	0 59 ± 0.18	0.66 ± 0.18	0.44 ± 0.12	0.45 ± 0.13	1.06 ± 0 05	1 ± 0.24
Opto-GPR88	0.52 ± 0.08	0.44 ± 0 04	0 55 ± 0.17	1.88 ± 0 92	0.48 ± 0.15	0 55 ± 0.19	1.04 ± 0 06	1.02 ± 0.12
Opto-GPR119	1.27 ± 0.45	0.87 ± 0 23	0 64 ± 0.17	0.67 ± 0.14	0.47 ± 0.09	0 52 ± 0.10	1.06 ± 0.14	0 9 ± 0.10
Opto-GPR120	0.69 ± 0.18	0,48 ± 0,20	1,78 ± 0,79	1,59 ± 1,02	1,22 ± 0,54	0,80 ± 0,64	0,65 ± 0,24	0,77 ± 0,19
Opto-GPR132	1.33 ± 0.43	0.66 ± 0 20	0 57 ± 0.18	0.55 ± 0.15	0.5 ± 0.11	0 69 ± 0.17	0.96 ± 0 09	0.89 ± 0.07
Opto-GPR135	0.57 ± 0.12	6.78 ± 3 09	0 64 ± 0.09	1.11 ± 0.45	0 58 ± 0.14	0 55 ± 0.14	1.07 ± 0.10	1.09 ± 0.23
Opto-GPR139	0.42 ± 0.09	0.43 ± 0.11	0.41 ± 0.08	0.53 ± 0.11	0.46 ± 0.11	0 55 ± 0.12	0.8 ± 0.03	1.18 ± 0.41
Opto-GPR141	0.55 ± 0.13	0.37 ± 0 08	0 58 ± 0.15	0.45 ± 0.12	0 63 ± 0.16	0 68 ± 0.20	0.93 ± 0 08	1.28 ± 0.41
Opto-GPR142	0.33 ± 0.12	0.3 ± 0.05	0 67 ± 0.29	0.46 ± 0.14	0.7 ± 0.17	0 91 ± 0.19	0.76 ± 0 04	0.95 ± 0.23
Opto-GPR146	0.65 ± 0.15	1.06 ± 0.43	0 96 ± 0.21	1.62 ± 0 89	0.6 ± 0.17	0 58 ± 0.12	1.5 ± 0.45	1.09 ± 0.24
Opto-GPR148	0.55 ± 0.13	0.78 ± 0 39	1 05 ± 0.29	0.68 ± 0 25	0.81 ± 0.4	0 58 ± 0.16	1.09 ± 0 07	0.95 ± 0.13
Opto-GPR149	0.46 ± 0.10	1.01 ± 0 39	0.88 ± 0 3	0.69 ± 0.17	0 66 ± 0.28	0 54 ± 0.18	1.21 ± 0.15	0.91 ± 0.19
Opto-GPR150	0.74 ± 0.26	1.05 ± 0 32	1.43 ± 0.31	1.26 ± 0 33	0.48 ± 0.12	0.7 ± 0.23	1.15 ± 0.10	0.73 ± 0.14
Opto-GPR151	0.52 ± 0.09	0.75 ± 0.18	0 65 ± 0.17	1.17 ± 0 25	0 87 ± 0.49	0.49 ± 0.12	0.83 ± 0 06	0 9 ± 0 09
Opto-GPR152	0.42 ± 0.08	0.48 ± 0.10	0 56 ± 0.17	0.74 ± 0 26	0 52 ± 0.10	0 68 ± 0.16	0.87 ± 0.13	0.88 ± 0.17
Opto-GPR153	0.67 ± 0.15	0.67 ± 0 09	0 66 ± 0.21	0.69 ± 0.18	0 54 ± 0.12	0.75 ± 0.19	0.93 ± 0.10	1.1 ± 0.16
Opto-GPR160	0.44 ± 0.04	0.58 ± 0.15	0 67 ± 0.26	0.86 ± 0 51	0 87 ± 0.13	0 83 ± 0.13	1.13 ± 0.12	1.02 ± 0.15
Opto-GPR161	0.57 ± 0.08	0.67 ± 0.13	0 66 ± 0.21	0.65 ± 0 21	0.7 ± 0.18	0.74 ± 0.20	0.88 ± 0.11	1.04 ± 0.25
Opto-GPR162	0 39 ± 0.1	0.57 ± 0 08	0 61 ± 0.13	0.88 ± 0 34	0.73 ± 0.14	0 95 ± 0.24	0.98 ± 0 06	1.68 ± 0.79
Opto-GPR171	0.54 ± 0.09	0.54 ± 0.11	0.70 ± 0.10	0.73 ± 0 21	0 63 ± 0.12	0.74 ± 0.13	1.08 ± 0 06	2.19 ± 1.20
Opto-GPR173	0.37 ± 0.09	0.38 ± 0 09	0 59 ± 0.14	0.57 ± 0.13	1 98 ± 1.18	0.8 ± 0.11	1.1 ± 0.06	1.46 ± 0.27
Opto-GPR174	1.01 ± 0.23	0.87 ± 0.19	1 28 ± 0.69	0.68 ± 0.16	0 55 ± 0.17	0.74 ± 0.09	1.14 ± 0 26	1.02 ± 0.23
Opto-GPR176	0.53 ± 0.12	0.64 ± 0.12	0.96 ± 0 2	0.72 ± 0.13	0 67 ± 0.22	0 65 ± 0.14	0.91 ± 0 06	1.02 ± 0.12

Opto-GPR182	0.71 ± 0.18	0.75 ± 0 32	0.7 ± 0.11	1.72 ± 0 96	0 52 ± 0.15	0 56 ± 0.11	0.98 ± 0 07	1.13 ± 0.12
Opto-GPR183	0.43 ± 0.09	0.65 ± 0 07	0 88 ± 0.16	2.02 ± 1 01	0.76 ± 0.23	0 68 ± 0.18	0.96 ± 0 08	0 91 ± 0.11

Table 6.2 Mean induction values for each light-activated human Class A oGPCRs for the different luciferase-based gene reporters used for the functional screening (section 4.2, 4.3)

7-Appendix II

GPR1

Knockout references

http://www.mousephenotype.org/data/genes/MGI:2385324 ;
http://www.informatics.jax.org/allele/MGI:4434954?page=alleleDetail&id=MGI:4434954

Phenotype references

- Hansen GM, Markesich DC, Burnett MB, Zhu Q, Dionne KM, Richter LJ, Finnell RH, Sands AT, Zambrowicz BP, Abuin A, Large-scale gene trapping in C57BL/6N mouse embryonic stem cells. Genome Res. 2008 Oct;18(10):1670-9

- Friedel RH, Seisenberger C, Kaloff C, Wurst W, EUCOMM--the European conditional mouse mutagenesis program. Brief Funct Genomic Proteomic. 2007 Sep;6(3):180-5

- Lin Q, Donahue SL, Moore-Jarrett T, Cao S, Osipovich AB, Ruley HE, Mutagenesis of diploid mammalian genes by gene entrapment. Nucleic Acids Res. 2006;34(20):e139

- Zambrowicz BP, Abuin A, Ramirez-Solis R, Richter LJ, Piggott J, BeltrandelRio H, Buxton EC, Edwards J, Finch RA, Friddle CJ, Gupta A, Hansen G, Hu Y, Huang W, Jaing C, Key BW Jr, Kipp P, Kohlhauff B, Ma ZQ, Markesich D, Payne R, Potter DG, Qian N, Shaw J, Schrick J, Shi ZZ, Sparks MJ, Van Sligtenhorst I, Vogel P, Walke W, Xu N, Zhu Q, Person C, Sands AT, Wnk1 kinase deficiency lowers blood pressure in mice: a gene-trap screen to identify potential targets for therapeutic intervention. Proc Natl Acad Sci U S A. 2003 Nov 25;100(24):14109-14

- Lyon MF, Rastan S, Brown SDM, in Lyon MF, Rastan S, Brown SDM (eds), Genetic Variants and Strains of the Laboratory Mouse. 3rd Edition. 1996Oxford: Oxford University Press.

- Juriloff DM, Sulik KK, Roderick TH, Hogan BK, Genetic and developmental studies of a new mouse mutation that produces otocephaly. J Craniofac Genet Dev Biol. 1985;5(2):121-45

- Roderick TH, Using inversions to detect and study recessive lethals and detrimentals in mice, in de Serres FJ, Sheridan W (eds), Utilization of Mammalian Specific Locus Studies in Hazard Evaluation and Estimation of Genetic Risk. 1983:135-67. Plenum Publishing Corp..

- Roderick TH, Hawes NL, Two radiation-induced chromosomal inversions in mice (Mus musculus). Proc Natl Acad Sci U S A. 1970 Oct;67(2):961-7

GPR2

Knockout references

http://www.mousephenotype.org/data/genes/MGI:2385324

http://www.informatics.jax.org/marker/MGI:1096320

Phenotype references

- Cai Y, Xue F, Fleming C, Yang J, Ding C, Ma Y, Liu M, Zhang HG, Zheng J, Xiong N, Yan J, Differential developmental requirement and peripheral regulation for dermal Vgamma4 and Vgamma6T17 cells in health and inflammation. Nat Commun. 2014;5:3986

- Agnello D, Denimal D, Lavaux A, Blondeau-Germe L, Lu B, Gerard NP, Gerard C, Pothier P, Intrarectal immunization and IgA antibody-secreting cell homing to the small intestine. J Immunol. 2013 May 1;190(9):4836-47

- Hu S, Yang K, Yang J, Li M, Xiong N, Critical roles of chemokine receptor CCR10 in regulating memory IgA responses in intestines. Proc Natl Acad Sci U S A. 2011 Nov 8;108(45):E1035-44

- Tubo NJ, McLachlan JB, Campbell JJ, Chemokine receptor requirements for epidermal T-cell trafficking. Am J Pathol. 2011 Jun;178(6):2496-503

- Terzian T, Dumble M, Arbab F, Thaller C, Donehower LA, Lozano G, Justice MJ, Roop DR, Box NF, Rpl27a mutation in the sooty foot ataxia mouse phenocopies high p53 mouse models. J Pathol. 2011 Aug;224(4):540-52

- Skarnes WC, Rosen B, West AP, Koutsourakis M, Bushell W, Iyer V, Mujica AO, Thomas M, Harrow J, Cox T, Jackson D, Severin J, Biggs P, Fu J, Nefedov M, de Jong PJ, Stewart AF, Bradley A, A conditional knockout resource for the genome-wide study of mouse gene function. Nature. 2011 Jun 16;474(7351):337- 42

- Xia M, Qi Q, Jin Y, Wiest DL, August A, Xiong N, Differential roles of IL-2-inducible T cell kinase-mediated TCR signals in tissue-specific localization and maintenance of skin intraepithelial T cells. J Immunol. 2010 Jun 15;184(12):6807-14

- Jiang X, Campbell JJ, Kupper TS, Embryonic trafficking of gammadelta T cells to skin is dependent on E/P selectin ligands and CCR4. Proc Natl Acad Sci U S A. 2010 Apr 20;107(16):7443-8

- Jin Y, Xia M, Sun A, Saylor CM, Xiong N, CCR10 Is Important for the Development of Skin-Specific {gamma}{delta}T Cells by Regulating Their Migration and Location. J Immunol. 2010 Oct 11;

- Lieberoth A, Splittstoesser F, Katagihallimath N, Jakovcevski I, Loers G, Ranscht B, Karagogeos D, Schachner M, Kleene R, Lewis(x) and alpha2,3-sialyl glycans and their receptors TAG-1, Contactin, and L1 mediate CD24-dependent neurite outgrowth. J Neurosci. 2009 May 20;29(20):6677-90

- Sousa AD, Andrade LR, Salles FT, Pillai AM, Buttermore ED, Bhat MA, Kachar B, The septate junction protein caspr is required for structural support and retention of KCNQ4 at calyceal synapses of vestibular hair cells. J Neurosci. 2009 Mar 11;29(10):3103-8

- Boles MK, Wi kinson BM, Wilming LG, Liu B, Probst FJ, Harrow J, Grafham D, Hentges KE, Woodward LP, Maxwell A, Mitchell K, Risley MD, Johnson R, Hirschi K, Lupski JR, Funato Y, Miki H, Marin-Garcia P, Matthews L, Coffey AJ, Parker A, Hubbard TJ, Rogers J,Bradley A, Adams DJ, Justice MJ, Discovery of candidate disease genes in ENU-induced mouse mutants by large-scale sequencing, including a splice-site mutation in nucleoredoxin. PLoS Genet. 2009 Dec;5(12):e1000759

- Morteau O, Gerard C, Lu B, Ghiran S, Rits M, Fujiwara Y, Law Y, Distelhorst K, Nielsen EM, Hill ED, Kwan R, Lazarus NH, Butcher EC, Wilson E, An indispensable role for the chemokine receptor CCR10 in IgA ant body-secreting cell accumulation. J Immunol. 2008 Nov 1;181(9):6309-15

- Pillai AM, Garcia-Fresco GP, Sousa AD, Dupree JL, Philpot BD, Bhat MA, No effect of genetic deletion of contactin-associated protein (CASPR) on axonal orientation and synaptic plasticity. J Neurosci Res. 2007 Aug 15;85(11):2318-31

- Garcia-Fresco GP, Sousa AD, Pillai AM, Moy SS, Crawley JN, Tessarollo L, Dupree JL, Bhat MA, Disruption of axo-glial junctions causes cytoskeletal disorganization and degeneration of Purkinje neuron axons. Proc Natl Acad Sci U S A. 2006 Mar 28;103(13):5137-42

- Clark AT, Firozi K, Justice MJ, Mutations in a novel locus on mouse chromosome 11 resulting in male infertility associated with defects in microtubule assembly and sperm tail function. Biol Reprod. 2004 May;70(5):1317-24

- Rios JC, Rubin M, St Martin M, Downey RT, Einheber S, Rosenbluth J, Levinson SR, Bhat M, Salzer JL, Paranodal interactions regulate expression of sodium channel subtypes and provide a diffusion barrier for the node of Ranvier. J Neurosci. 2003 Aug 6;23(18):7001-11

- Kile BT, Hentges KE, Clark AT, Nakamura H, Salinger AP, Liu B, Box N, Stockton DW, Johnson RL, Behringer RR, Bradley A, Justice MJ, Functional genetic analysis of mouse chromosome 11. Nature. 2003 Sep 4;425(6953):81-6

- Bhat MA, Rios JC, Lu Y, Garcia-Fresco GP, Ching W, Martin MS, Li J, Einheber S, Chesler M, Rosenbluth J, Salzer JL, Bellen HJ, Axon-glia interactions and the domain organization of myelinated axons requires neurexin iv/caspr/paranodin. Neuron. 2001 May;30(2):369-83

- Zheng B, Sage M, Cai WW, Thompson DM, Tavsanli BC, Cheah YC, Bradley A, Engineering a mouse balancer chromosome. Nat Genet. 1999 Aug;22(4):375-8

GPR3

Knockout references

http://www.mousephenotype.org/data/genes/MGI:101908

Phenotype references

- Tanaka S, Miyagi T, Dohi E, Seki T, Hide I, Sotomaru Y, Saeki Y, Antonio Chiocca E, Matsumoto M, Sakai N, Developmental expression of GPR3 in rodent cerebellar granule neurons is associated with cell survival and protects neurons from various apoptotic stimuli. Neurobiol Dis. 2014 Aug;68:215-27

- Su YQ, Sugiura K, Sun F, Pendola JK, Cox GA, Handel MA, Schimenti JC, Eppig JJ, MARF1 regulates essential oogenic processes in mice. Science. 2012 Mar 23;335(6075):1496-9

- Tanaka S, Sha kh IM, Chiocca EA, Saeki Y, The Gs-linked receptor GPR3 inhibits the proliferation of cerebellar granule cells during postnatal development. PLoS One. 2009;4(6):e5922

- Thathiah A, Spittaels K, Hoffmann M, Staes M, Cohen A, Horre K, Vanbrabant M, Coun F, Baekelandt V, Delacourte A, Fischer DF, Pollet D, De Strooper B, Merchiers P, The orphan G protein-coupled receptor 3 modulates amyloid-beta peptide generation in neurons. Science. 2009 Feb 13;323(5916):946-51

- Valverde O, Celerier E, Baranyi M, Vanderhaeghen P, Maldonado R, Sperlagh B, Vassart G, Ledent C, GPR3 receptor, a novel actor in the emotional-l ke responses. PLoS One. 2009;4(3):e4704

- Vaccari S, Horner K, Mehlmann LM, Conti M, Generation of mouse oocytes defective in cAMP synthesis and degradation: endogenous cyclic AMP is essential for meiotic arrest. Dev Biol. 2008 Apr 1;316(1):124-34

- Freudzon L, Norris RP, Hand AR, Tanaka S, Saeki Y, Jones TL, Rasenick MM, Berlot CH, Mehlmann LM, Jaffe LA, Regulation of meiotic prophase arrest in mouse oocytes by GPR3, a constitutive activator of the Gs G protein. J Cell Biol. 2005 Oct24;171(2):255-65

- Ledent C, Demeestere I, Blum D, Petermans J, Hamalainen T, Smits G, Vassart G, Premature ovarian aging in mice deficient for Gpr3. Proc Natl Acad Sci U S A. 2005 Jun 21;102(25):8922-6

- Mehlmann LM, Saeki Y, Tanaka S, Brennan TJ, Evsikov AV, Pendola FL, Knowles BB, Eppig JJ, Jaffe LA, The Gs-linked receptor GPR3 maintains meiotic arrest in mammalian oocytes. Science. 2004 Dec 10;306(5703):1947-50

- Nishijima I, Mills A, Qi Y, Mills M, Bradley A, Two new balancer chromosomes on mouse chromosome 4 to facilitate functional annotation of human chromosome 1p. Genesis. 2003 Jul;36(3):142-8

- Lyon MF, Rastan S, Brown SDM, in Lyon MF, Rastan S, Brown SDM (eds), Genetic Variants and Strains of the Laboratory Mouse. 3rd Edition. 1996Oxford: Oxford University Press.

- Davisson MT, New chromosome aberrations. Mouse News Lett. 1988;80:166

- Roderick TH, Using inversions to detect and study recessive lethals and detrimentals in mice, in de Serres FJ, Sheridan W (eds), Utilization of Mammalian Specific Locus Studies in Hazard Evaluation and Estimation of Genetic Risk. 1983:135-67. Plenum Publishing Corp..

GPR4

Knockout references

http://www.mousephenotype.org/data/genes/MGI:2441992
http://www.informatics.jax.org/marker/MGI:2441992

Phenotype references

- Skarnes WC, Rosen B, West AP, Koutsourakis M, Bushell W, Iyer V, Mujica AO, Thomas M, Harrow J, Cox T, Jackson D, Severin J, Biggs P, Fu J, Nefedov M, de Jong PJ, Stewart AF, Bradley A, A conditional knockout resource for the genome-wide study of mouse gene function. Nature. 2011 Jun 16;474(7351):337- 42

- Sun X, Yang LV, Tiegs BC, Arend LJ, McGraw DW, Penn RB, Petrovic S, Deletion of the pH sensor GPR4 decreases renal acid excretion. J Am Soc Nephrol. 2010 Oct;21(10):1745-55

- Hansen GM, Markesich DC, Burnett MB, Zhu Q, Dionne KM, Richter LJ, Finnell RH, Sands AT, Zambrowicz BP, Abuin A, Large-scale gene trapping in C57BL/6N mouse embryonic stem cells. Genome Res. 2008 Oct;18(10):1670-9

- Yang LV, Radu CG, Roy M, Lee S, McLaughlin J, Teitell MA, Iruela-Arispe ML, Witte ON, Vascular abnormalities in mice deficient for the G protein-coupled receptor GPR4 that functions as a pH sensor. Mol Cell Biol. 2007 Feb;27(4):1334-47

- Zambrowicz BP, Abuin A, Ramirez-Solis R, Richter LJ, Piggott J, BeltrandelRio H, Buxton EC, Edwards J, Finch RA, Friddle CJ, Gupta A, Hansen G, Hu Y, Huang W, Jaing C, Key BW Jr, Kipp P, Kohlhauff B, Ma ZQ, Markesich D, Payne R, Potter DG, Qian N, Shaw J, Schrick J, Shi ZZ, Sparks MJ, Van Sligtenhorst I, Vogel P, Walke W, Xu N, Zhu Q, Person C, Sands AT, Wnk1 kinase deficiency lowers blood pressure in mice: a gene-trap screen to identify potential targets for therapeutic intervention. Proc Natl Acad Sci U S A. 2003 Nov 25;100(24):14109-14

GPR6

Knockout references

http://www.mousephenotype.org/data/genes/MGI:2155249
http://www.informatics.jax.org/marker/MGI:2155249

Phenotype references

- Oeckl P, Hengerer B, Ferger B, G-protein coupled receptor 6 deficiency alters striatal dopamine and cAMP concentrations and reduces dyskinesia in a mouse model of Parkinson's disease. Exp Neurol. 2014 Jul;257:1-9

- Skarnes WC, Rosen B, West AP, Koutsourakis M, Bushell W, Iyer V, Mujica AO, Thomas M, Harrow J, Cox T, Jackson D, Severin J, Biggs P, Fu J, Nefedov M, de Jong PJ, Stewart AF, Bradley A, A conditional knockout resource for the genome-wide study of mouse gene function. Nature. 2011 Jun 16;474(7351):337- 42

- Lobo MK, Cui Y, Ostlund SB, Balleine BW, William Yang X, Genetic control of instrumental conditioning by striatopallidal neuron-specific S1P receptor Gpr6. Nat Neurosci. 2007 Nov;10(11):1395-1397

- Benson KF, Chada K, Molecular Characterization of the Mouse In(10)17Rk Inversion and Identification of a Novel Muscle-Specific Gene at the Proximal Breakpoint. Genetics. 2002 Jan;160(1):279-87

- Lyon MF, Rastan S, Brown SDM, in Lyon MF, Rastan S, Brown SDM (eds), Genetic Variants and Strains of the Laboratory Mouse. 3rd Edition. 1996Oxford: Oxford University Press.

- Zhou X, Benson KF, Ashar HR, Chada K, Mutation respons ble for the mouse pygmy phenotype in the developmentally regulated factor HMGI-C [see comments]. Nature. 1995 Aug 31;376(6543):771-4

- Roderick TH, Using inversions to detect and study recessive lethals and detrimentals in mice, in de Serres FJ, Sheridan W (eds), Utilization of Mammalian Specific Locus Studies in Hazard Evaluation and Estimation of Genetic Risk. 1983:135-67. Plenum Publishing Corp..

- Roderick TH, Chromosomal inversions in studies of mammalian mutagenesis. Genetics. 1979 May;92(1 Pt 1 Suppl):s121-6

- Roderick TH, Hawes NL, Nineteen paracentric chromosomal inversions in mice. Genetics. 1974 Jan;76(1):109-17

GPR7

Knockout references

http://www.mousephenotype.org/data/genes/MGI:891989
http://www.informatics.jax.org/marker/MGI:891989

Phenotype references

- Nagata-Kuroiwa R, Furutani N, Hara J, Hondo M, Ishii M, Abe T, Mieda M, Tsujino N, Motoike T, Yanagawa Y, Kuwaki T, Yamamoto M, Yanagisawa M, Sakurai T, Critical role of neuropeptides b/w receptor 1 signaling in social behavior and fear memory. PLoS One. 2011;6(2):e16972

- Ishii M, Fei H, Friedman JM, Targeted disruption of GPR7, the endogenous receptor for neuropeptides B and W, leads to metabolic defects and adult-onset obesity. Proc Natl Acad Sci U S A. 2003 Sep 2;100(18):10540-5

GPR14

Knockout references

http://www.mousephenotype.org/data/genes/MGI:2183450

Phenotype references

- Skarnes WC, Rosen B, West AP, Koutsourakis M, Bushell W, Iyer V, Mujica AO, Thomas M, Harrow J, Cox T, Jackson D, Severin J, Biggs P, Fu J, Nefedov M, de Jong PJ, Stewart AF, Bradley A, A conditional knockout resource for the genome-wide study of mouse gene function. Nature. 2011 Jun 16;474(7351):337- 42

- Bousette N, D'Orleans-Juste P, Kiss RS, You Z, Genest J, Al-Ramli W, Qureshi ST, Gramolini A, Behm D, Ohlstein EH, Harrison SM, Douglas SA, Giaid A, Urotensin II receptor knockout mice on an ApoE knockout background fed a high-fat diet exhibit an enhanced hyperlipidemic and atherosclerotic phenotype. Circ Res. 2009 Sep 25;105(7):686-95, 19 p following 695

- Behm DJ, Harrison SM, Ao Z, Maniscalco K, Pickering SJ, Grau EV, Woods TN, Coatney RW, Doe CP, Willette RN, Johns DG, Douglas SA, Deletion of the UT receptor gene results in the selective loss of urotensin-II contractile activity in aortae isolated from UT receptor knockout mice. Br J Pharmacol. 2003 May;139(2):464-72

GPR17

Knockout references

http://www.mousephenotype.org/data/genes/MGI:3584514

Phenotype references

- Mastaitis J, Min S, Elvert R, Kannt A, Xin Y, Ochoa F, Gale NW, Valenzuela DM, Murphy AJ, Yancopoulos GD, Gromada J, GPR17 gene disruption does not alter food intake or glucose homeostasis in mice. Proc Natl Acad Sci U S A. 2015 Feb 10;112(6):1845-9

- Maekawa A, Xing W, Austen KF, Kanaoka Y, GPR17 regulates immune pulmonary inflammation induced by house dust mites. J Immunol. 2010 Aug 1;185(3):1846-54

- Maekawa A, Balestrieri B, Austen KF, Kanaoka Y, GPR17 is a negative regulator of the cysteinyl leukotriene 1 receptor response to leukotriene D4. Proc Natl Acad Sci U S A. 2009 Jul 14;106(28):11685-90

- Chen Y, Wu H, Wang S, Koito H, Li J, Ye F, Hoang J, Escobar SS, Gow A, Arnett HA, Trapp BD, Karandikar NJ, Hsieh J, Lu QR, The oligodendrocyte-specific G protein-coupled receptor GPR17 is a cell-intrinsic timer of myelination. Nat Neurosci. 2009 Nov;12(11):1398-406

GPR18

Knockout references

http://www.mousephenotype.org/data/genes/MGI:107859

Phenotype references

Velocigene, Alleles produced for the KOMP project by Velocigene (Regeneron Pharmaceuticals). MGI Direct Data Submission. 2008

GPR19

Knockout references

http://www.mousephenotype.org/data/genes/MGI:892973

Phenotype references

- Hansen GM, Markesich DC, Burnett MB, Zhu Q, Dionne KM, Richter LJ, Finnell RH, Sands AT, Zambrowicz BP, Abuin A, Large-scale gene trapping in C57BL/6N mouse embryonic stem cells. Genome Res. 2008 Oct;18(10):1670-9

- Friedel RH, Seisenberger C, Kaloff C, Wurst W, EUCOMM--the European conditional mouse mutagenesis program. Brief Funct Genomic Proteomic. 2007 Sep;6(3):180-5

- Stryke D, Kawamoto M, Huang CC, Johns SJ, King LA, Harper CA, Meng EC, Lee RE, Yee A, L'Italien L, Chuang PT, Young SG, Skarnes WC, Babbitt PC, Ferrin TE, BayGenomics: a resource of insertional mutations in mouse embryonic stem cells. Nucleic Acids Res. 2003 Jan 1;31(1):278-81

- BP, Abuin A, Ramirez-Solis R, Richter LJ, Piggott J, BeltrandelRio H, Buxton EC, Edwards J, Finch RA, Friddle CJ, Gupta A, Hansen G, Hu Y, Huang W, Jaing C, Key BW Jr, Kipp P, Kohlhauff B, Ma ZQ, Markesich D, Payne R, Potter DG, Qian N, Shaw J, Schrick J, Shi ZZ, Sparks MJ, Van Sligtenhorst I, Vogel P, Walke W, Xu N, Zhu Q, Person C, Sands AT, Wnk1 kinase deficiency lowers blood pressure in mice: a gene-trap screen to identify potential targets for therapeutic intervention. Proc Natl Acad Sci U S A. 2003 Nov 25;100(24):14109-14

GPR20

Knockout references

http://www.mousephenotype.org/data/genes/MGI:2441803
http://www.informatics.jax.org/marker/MGI:2441803

Phenotype references

- Skarnes WC, Rosen B, West AP, Koutsourakis M, Bushell W, Iyer V, Mujica AO, Thomas M, Harrow J, Cox T, Jackson D, Severin J, Biggs P, Fu J, Nefedov M, de Jong PJ, Stewart AF, Bradley A, A conditional knockout resource for the genome-wide study of mouse gene function. Nature. 2011 Jun 16;474(7351):337- 42

- Katayama K, Miyamoto S, Furuno A, Akiyama K, Takahashi S, Suzuki H, Tsuji T, Kunieda T, Characterization of the chromosomal inversion associated with the Koa mutation in the mouse revealed the cause of skeletal abnormalities. BMC Genet. 2009;10:60

- Hansen GM, Markesich DC, Burnett MB, Zhu Q, Dionne KM, Richter LJ, Finnell RH, Sands AT, Zambrowicz BP, Abuin A, Large-scale gene trapping in C57BL/6N mouse embryonic stem cells. Genome Res. 2008 Oct;18(10):1670-9

- Mentzer SE, Sundberg JP, Awgulewitsch A, Chao HH, Carpenter DA, Zhang WD, Rinch k EM, You Y, The mouse hairy ears mutation exhibits an extended growth (anagen) phase in hair follicles and altered Hoxc gene expression in the ears. Vet Dermatol. 2008 Dec;19(6):358-67

- Fantauzzo KA, Tadin-Strapps M, You Y, Mentzer SE, Baumeister FA, Cianfarani S, Van Maldergem L, Warburton D, Sundberg JP, Christiano AM, A position effect on TRPS1 is associated with Ambras syndrome in humans and the Koala phenotype in mice. Hum Mol Genet. 2008 Nov 15;17(22):3539-51

- Katayama K, Furuno A, Akiyama K, Tsuji T, Kunieda T, Characterization of chromosomal inversion of the mouse hairy ears (Eh) mutation associated with cleft palate. Mamm Genome. 2007 Apr;18(4):246-54

- Cook MN, Dunning JP, Wiley RG, Chesler EJ, Johnson DK, Miller DR, Goldowitz D, Neurobehavioral mutants identified in an ENU-mutagenesis project. Mamm Genome. 2007 Aug;18(8):559-72

- Peters J, Ball ST, Biochemical and recessive visible specific locus responses of C3H/HeH to fractionated, acute radiation. Mutat Res. 2003 Mar;543(2):137-43

- Lyon MF, Rastan S, Brown SDM, in Lyon MF, Rastan S, Brown SDM (eds), Genetic Variants and Strains of the Laboratory Mouse. 3rd Edition. 1996Oxford: Oxford University Press.

- Hogan ME, King LE Jr, Sundberg JP, Defects of pelage hairs in 20 mouse mutations. J Invest Dermatol. 1995 May;104(5 Suppl):31S-32S

- Sundberg JP (ed.), Handbook of Mouse Mutations with Skin and Hair Abnormalities: Animal Models and Biomedical Tools, in John P. Sundberg, ed. (eds), Handbook of Mouse Mutations with Skin and Hair Abnormalities: Animal Models and Biomedical Tools. 1994Boca Raton: CRC Press.

- Peters J, Tease C, Ball ST, Koala, Koa, is associated with an inversion on mouse Chromosome 15. Genet Res. 1992;59:237-8 (Abstract)

- Davisson MT, Roderick TH, Akeson EC, Hawes NL, Sweet HO, The hairy ears (Eh) mutation is closely associated with a chromosomal rearrangement in mouse chromosome 15. Genet Res. 1990 Oct-Dec;56(2-3):167-78

- Ball ST, Peters J, Koala, a dominant mutation. Mouse News Lett. 1989;83:163-4

- Searle AG, Beechey CV, Map of reciprocal translocations, inversions, and insertions, in MF Lyon, AG Searle (eds), Genetic Variants and Strains of the Laboratory Mouse. 1989:620-5. Oxford, UK: Oxford University Press.

- Lane PW, Liu HM, Association of megacolon with a new dominant spotting gene (Dom) in the mouse. J Hered. 1984 Nov-Dec;75(6):435-9

- Roderick TH, Using inversions to detect and study recessive lethals and detrimentals in mice, in de Serres FJ, Sheridan W (eds), Utilization of Mammalian Specific Locus Studies in Hazard Evaluation and Estimation of Genetic Risk. 1983:135-67. Plenum Publishing Corp..

- Roderick TH, Chromosomal inversions in studies of mammalian mutagenesis. Genetics. 1979 May;92(1 Pt 1 Suppl):s121-6

- Guenet JL, Hairy ears (Eh). Mouse News Lett. 1978;58:68

- Guenet JL, Aggregation chimeras between normal and recessive lethal genotypes. Mouse News Lett. 1978;58:67

- Roderick TH, Hawes NL, Nineteen paracentric chromosomal inversions in mice. Genetics. 1974 Jan;76(1):109-17

- Bangham JW, Hairy ears, Eh. Mouse News Lett. 1968;38:32

- Bangham JW, Hairy ears, Eh. Mouse News Lett. 1965;33:68

GPR21

Knockout references

http://www.mousephenotype.org/data/genes/MGI:2441890
http://www.informatics.jax.org/marker/MGI:2441890

Phenotype references

- Osborn O, Oh DY, McNelis J, Sanchez-Alavez M, Talukdar S, Lu M, Li P, Thiede L, Morinaga H, Kim JJ, Heinrichsdorff J, Nalbandian S, Ofrecio JM, Scadeng M, Schenk S, Hadcock J, Bartfai T, Olefsky JM, G protein-coupled receptor 21 deletion improves insulin sensitivity in diet-induced obese mice. J Clin Invest. 2012 Jul 2;122(7):2444-53

- Gardner J, Wu S, Ling L, Danao J, Li Y, Yeh WC, Tian H, Baribault H, G-protein-coupled receptor GPR21 knockout mice display improved glucose tolerance and increased insulin response. Biochem Biophys Res Commun. 2012 Feb 3;418(1):1-5

GPR22

Knockout references

http://www.mousephenotype.org/data/genes/MGI:1920260;
http://www.informatics.jax.org/allele/MGI:4362775?page=alleleDetail&id=MGI:4362775

Phenotype references

- Skarnes WC, Rosen B, West AP, Koutsourakis M, Bushell W, Iyer V, Mujica AO, Thomas M, Harrow J, Cox T, Jackson D, Severin J, Biggs P, Fu J, Nefedov M, de Jong PJ, Stewart AF, Bradley A, A conditional knockout resource for the genome-wide study of mouse gene function. Nature. 2011 Jun 16;474(7351):337- 42

- Adams JW, Wang J, Davis JR, Liaw C, Gaidarov I, Gatlin J, Dalton ND, Gu Y, Ross J Jr, Behan D, Chien K, Connolly D, Myocardial expression, signaling, and function of GPR22: a protective role for an orphan G protein-coupled receptor. Am J Physiol Heart Circ Physiol. 2008 Aug;295(2):H509-21

GPR23

Knockout references

http://www.informatics.jax.org/marker/MGI:1925384

Phenotype references

- Sumida H, Noguchi K, Kihara Y, Abe M, Yanagida K, Hamano F, Sato S, Tamaki K, Morishita Y, Kano MR, Iwata C, Miyazono K, Sakimura K, Shimizu T, Ishii S, LPA4 regulates blood and lymphatic vessel formation during mouse embryogenesis. Blood. 2010 Dec 2;116(23):5060-70

- Hansen GM, Markesich DC, Burnett MB, Zhu Q, Dionne KM, Richter LJ, Finnell RH, Sands AT, Zambrowicz BP, Abuin A, Large-scale gene trapping in C57BL/6N mouse embryonic stem cells. Genome Res. 2008 Oct;18(10):1670-9

GPR24

Knockout references

http://www.mousephenotype.org/data/genes/MGI:2180756
http://www.informatics.jax.org/marker/MGI:2180756

Phenotype references

- Chee MJ, Pissios P, Maratos-Flier E, Neurochemical charaterization of neurons expressing melanin-concentrating hormone receptor 1 in the mouse hypothalamus. J Comp Neurol. 2013 Jul;521(10):2208-34

- Jego S, Glasgow SD, Herrera CG, Ekstrand M, Reed SJ, Boyce R, Friedman J, Burdakov D, Adamantidis AR, Optogenetic identification of a rapid eye movement sleep modulatory circuit in the hypothalamus. Nat Neurosci. 2013 Nov;16(11):1637-43

- Conductier G, Brau F, Viola A, Langlet F, Ramkumar N, Dehouck B, Lemaire T, Chapot R, Lucas L, Rovere C, Maitre P, Hosseiny S, Petit-Paitel A, Adamantidis A, Lakaye B, Risold PY, Prevot V, Meste O, Nahon JL, Guyon A, Melanin-concentrating hormone regulates beat frequency of ependymal cilia and ventricular volume. Nat Neurosci. 2013 Jul;16(7):845-7

- Chung S, Liao XH, Di Cosmo C, Van Sande J, Wang Z, Refetoff S, Civelli O, Disruption of the melanin-concentrating hormone receptor 1 (MCH1R) affects thyroid function. Endocrinology. 2012 Dec;153(12):6145-54

- Parks GS, Okumura SM, Gohil K, Civelli O, Mice lacking Melanin Concentrating Hormone 1 receptor are resistant to seizures. Neurosci Lett. 2010 Oct 29;484(2):104-7

- Chung S, Hopf FW, Nagasaki H, Li CY, Belluzzi JD, Bonci A, Civelli O, The melanin-concentrating hormone system modulates cocaine reward. Proc Natl Acad Sci U S A. 2009 Apr 21;106(16):6772-7

- Katayama K, Miyamoto S, Furuno A, Akiyama K, Takahashi S, Suzuki H, Tsuji T, Kunieda T, Characterization of the chromosomal inversion associated with the Koa mutation in the mouse revealed the cause of skeletal abnormalities. BMC Genet. 2009;10:60

- Adamantidis A, Salvert D, Goutagny R, Lakaye B, Gervasoni D, Grisar T, Luppi PH, Fort P, Sleep architecture of the melanin-concentrating hormone receptor 1-knockout mice. Eur J Neurosci. 2008 Apr;27(7):1793-800

- Tyhon A, Lakaye B, Adamantidis A, Tirelli E, Amphetamine- and cocaine-induced conditioned place preference and concomitant psychomotor sensitization in mice with genetically inactivated melanin- concentrating hormone MCH(1) receptor. Eur J Pharmacol. 2008 Dec 3;599(1-3):72-80

- Mentzer SE, Sundberg JP, Awgulewitsch A, Chao HH, Carpenter DA, Zhang WD, Rinchik EM, You Y, The mouse hairy ears mutation exh bits an extended growth (anagen) phase in hair follicles and altered Hoxc gene expression in the ears. Vet Dermatol. 2008 Dec;19(6):358-67

- Fantauzzo KA, Tadin-Strapps M, You Y, Mentzer SE, Baumeister FA, Cianfarani S, Van Maldergem L, Warburton D, Sundberg JP, Christiano AM, A position effect on TRPS1 is associated with Ambras syndrome in humans and the Koala phenotype in mice. Hum Mol Genet. 2008 Nov 15;17(22):3539-51

- Brommage R, Desai U, Revelli JP, Donoviel DB, Fontenot GK, Dacosta CM, Smith DD, Kirkpatrick LL, Coker KJ, Donoviel MS, Eberhart DE, Holt KH, Kelly MR, Paradee WJ, Philips AV, Platt KA, Suwanichkul A, Hansen GM, Sands AT, Zambrowicz BP, Powell DR, High-throughput screening of mouse knockout lines identifies true lean and obese phenotypes. Obesity (Silver Spring). 2008 Oct;16(10):2362-7

- Katayama K, Furuno A, Akiyama K, Tsuji T, Kunieda T, Characterization of chromosomal inversion of the mouse hairy ears (Eh) mutation associated with cleft palate. Mamm Genome. 2007 Apr;18(4):246-54

- Lalonde R, Qian S, Exploratory activity, motor coordination, and spatial learning in Mchr1 knockout mice. Behav Brain Res. 2007 Mar 28;178(2):293-304

- Cook MN, Dunning JP, Wiley RG, Chesler EJ, Johnson DK, Miller DR, Goldowitz D, Neurobehavioral mutants identified in an ENU-mutagenesis project. Mamm Genome. 2007 Aug;18(8):559-72

- Roy M, David N, Cueva M, Giorgetti M, A study of the involvement of melanin-concentrating hormone receptor 1 (MCHR1) in murine models of depression. Biol Psychiatry. 2007 Jan 15;61(2):174-80

- Bjursell M, Gerdin AK, Ploj K, Svensson D, Svensson L, Oscarsson J, Snaith M, Tornell J, Bohlooly-Y M, Melanin-concentrating hormone receptor 1 deficiency increases insulin sensitivity in obese leptin-deficient mice without affecting body weight. Diabetes. 2006 Mar;55(3):725-33

- Adamantidis A, Foidart A, Grisar T, Lakaye B, Tirelli E, Mice lacking the melanin-concentrating hormone receptor-1 exhibit an atypical psychomotor susceptibility to cocaine and no conditioned cocaine response. Behav Brain Res. 2006 Oct 2;173(1):94-103

- Roy M, David NK, Danao JV, Baribault H, Tian H, Giorgetti M, Genetic inactivation of melanin-concentrating hormone receptor subtype 1 (MCHR1) in mice exerts anxiolytic-like behavioral effects. Neuropsychopharmacology. 2006 Jan;31(1):112-20

- Egecioglu E, Gerdin AK, Svensson L, Oscarsson J, Morgan D, Snaith M, Tornell J, Bohlooly-Y M, Importance of melanin-concentrating hormone receptor for the acute effects of ghrelin. Biochem Biophys Res Commun. 2005 Jan 28;326(4):759-65

- Smith DG, Tzavara ET, Shaw J, Luecke S, Wade M, Davis R, Salhoff C, Nomikos GG, Gehlert DR, Mesolimbic dopamine super-sensitivity in melanin-concentrating hormone-1 receptor-deficient mice. J Neurosci. 2005 Jan 26;25(4):914-22

- Adamantidis A, Thomas E, Foidart A, Tyhon A, Coumans B, Minet A, Tirelli E, Seutin V, Grisar T, Lakaye B, Disrupting the melanin-concentrating hormone receptor 1 in mice leads to cognitive deficits and alterations of NMDA receptor function. Eur J Neurosci. 2005 May;21(10):2837-44

- Zhou D, Shen Z, Strack AM, Marsh DJ, Shearman LP, Enhanced running wheel activity of both Mch1r- and Pmch-deficient mice. Regul Pept. 2005 Jan 15;124(1-3):53-63

- Astrand A, Bohlooly-Y M, Larsdotter S, Mahlapuu M, Andersen H, Tornell J, Ohlsson C, Snaith M, Morgan DG, Mice lacking melanin-concentrating hormone receptor 1 demonstrate increased heart rate associated with altered autonomic activity. Am J Physiol Regul Integr Comp Physiol. 2004 Oct;287(4):R749-58

- Bohlooly-Y M, Mahlapuu M, Andersen H, Astrand A, Hjorth S, Svensson L, Tornell J, Snaith MR, Morgan DG, Ohlsson C, Osteoporosis in MCHR1-deficient mice. Biochem Biophys Res Commun. 2004 Jun 11;318(4):964-9

- Peters J, Ball ST, Biochemical and recessive visible specific locus responses of C3H/HeH to fractionated, acute radiation. Mutat Res. 2003 Mar;543(2):137-43

- Marsh DJ, Weingarth DT, Novi DE, Chen HY, Trumbauer ME, Chen AS, Guan XM, Jiang MM, Feng Y, Camacho RE, Shen Z, Frazier EG, Yu H, Metzger JM, Kuca SJ, Shearman LP, Gopal-Truter S, MacNeil DJ, Strack AM, MacIntyre DE, Van der Ploeg LH, Qian S, Melanin-concentrating hormone 1 receptor-deficient mice are lean, hyperactive, and hyperphagic and have altered metabolism. Proc Natl Acad Sci U S A. 2002 Mar 5;99(5):3240-5

- Chen Y, Hu C, Hsu CK, Zhang Q, Bi C, Asnicar M, Hsiung HM, Fox N, Slieker LJ, Yang DD, Heiman ML, Shi Y, Targeted disruption of the melanin-concentrating hormone receptor-1 results in hyperphagia and resistance to diet-induced obesity. Endocrinology. 2002 Jul;143(7):2469-77

- Lyon MF, Rastan S, Brown SDM, in Lyon MF, Rastan S, Brown SDM (eds), Genetic Variants and Strains of the Laboratory Mouse. 3rd Edition. 1996Oxford: Oxford University Press.

- Hogan ME, King LE Jr, Sundberg JP, Defects of pelage hairs in 20 mouse mutations. J Invest Dermatol. 1995 May;104(5 Suppl):31S-32S

- Sundberg JP (ed.), Handbook of Mouse Mutations with Skin and Hair Abnormalities: Animal Models and Biomedical Tools, in John P. Sundberg, ed. (eds), Handbook of Mouse Mutations with Skin and Hair Abnormalities: Animal Models and Biomedical Tools. 1994Boca Raton: CRC Press.

- Peters J, Tease C, Ball ST, Koala, Koa, is associated with an inversion on mouse Chromosome 15. Genet Res. 1992;59:237-8 (Abstract)

- Davisson MT, Roderick TH, Akeson EC, Hawes NL, Sweet HO, The hairy ears (Eh) mutation is closely associated with a chromosomal rearrangement in mouse chromosome 15. Genet Res. 1990 Oct-Dec;56(2-3):167-78

- Ball ST, Peters J, Koala, a dominant mutation. Mouse News Lett. 1989;83:163-4

- Lane PW, Liu HM, Association of megacolon with a new dominant spotting gene (Dom) in the mouse. J Hered. 1984 Nov-Dec;75(6):435-9

- Roderick TH, Using inversions to detect and study recessive lethals and detrimentals in mice, in de Serres FJ, Sheridan W (eds), Utilization of Mammalian Specific Locus Studies in Hazard Evaluation and Estimation of Genetic Risk. 1983:135-67. Plenum Publishing Corp..

- Guenet JL, Aggregation chimeras between normal and recessive lethal genotypes. Mouse News Lett. 1978;58:67

- Bangham JW, Hairy ears, Eh. Mouse News Lett. 1968;38:32

- Bangham JW, Hairy ears, Eh. Mouse News Lett. 1965;33:68

GPR26

Knockout references

http://www.mousephenotype.org/data/genes/MGI:2441758
http://www.informatics.jax.org/marker/MGI:2441758

Phenotype references

- Chen D, Liu X, Zhang W, Shi Y, Targeted inactivation of GPR26 leads to hyperphagia and adiposity by activating AMPK in the hypothalamus. PLoS One. 2012;7(7):e40764
- Zhang LL, Wang JJ, Liu Y, Lu XB, Kuang Y, Wan YH, Chen Y, Yan HM, Fei J, Wang ZG, GPR26-deficient mice display increased anxiety- and depression-like behaviors accompanied by reduced phosphorylated cyclic AMP responsive element-binding protein level in central amygdala. Neuroscience. 2011 Nov 24;196:203-14
- Skarnes WC, Rosen B, West AP, Koutsourakis M, Bushell W, Iyer V, Mujica AO, Thomas M, Harrow J, Cox T, Jackson D, Severin J, Biggs P, Fu J, Nefedov M, de Jong PJ, Stewart AF, Bradley A, A conditional knockout resource for the genome-wide study of mouse gene function. Nature. 2011 Jun 16;474(7351):337- 42

GPR30

Knockout references

http://www.mousephenotype.org/data/genes/MGI:1924104

Phenotype references

- Marjon NA, Hu C, Hathaway HJ, Prossnitz ER, G protein-coupled estrogen receptor regulates mammary tumorigenesis and metastasis. Mol Cancer Res. 2014 Nov;12(11):1644-54
- Meoli L, Isensee J, Zazzu V, Nabzdyk CS, Soewarto D, Witt H, Foryst-Ludwig A, Kintscher U, Noppinger PR, Sex- and age-dependent effects of Gpr30 genetic deletion on the metabolic and cardiovascular profiles of diet-induced obese mice. Gene. 2014 May 1;540(2):210-6
- Sharma G, Hu C, Brigman JL, Zhu G, Hathaway HJ, Prossnitz ER, GPER deficiency in male mice results in insulin resistance, dyslipidemia, and a proinflammatory state. Endocrinology. 2013 Nov;154(11):4136-45
- Hofmeister MV, Damkier HH, Christensen BM, Olde B, Fredrik Leeb-Lundberg LM, Fenton RA, Praetorius HA, Praetorius J, 17beta-Estradiol induces nongenomic effects in renal intercalated cells through G protein- coupled estrogen receptor 1. Am J Physiol Renal Physiol. 2012 Feb;302(3):F358-68
- Sharma G, Prossnitz ER, Mechanisms of Estradiol-Induced Insulin Secretion by the G Protein-Coupled Estrogen Receptor GPR30/GPER in Pancreatic {beta}-Cells. Endocrinology. 2011 Aug;152(8):3030-9
- Schackmann RC, van Amersfoort M, Haarhuis JH, Vlug EJ, Halim VA, Roodhart JM, Vermaat JS, Voest EE, van der Groep P, van Diest PJ, Jonkers J, Derksen PW, Cytosolic p120-catenin regulates growth of metastatic lobular carcinoma through Rock1-mediated anoikis resistance. J Clin Invest. 2011 Aug 1;121(8):3176-88
- Skarnes WC, Rosen B, West AP, Koutsourakis M, Bushell W, Iyer V, Mujica AO, Thomas M, Harrow J, Cox T, Jackson D, Severin J, Biggs P, Fu J, Nefedov M, de Jong PJ, Stewart AF, Bradley A, A conditional knockout resource for the genome-wide study of mouse gene function. Nature. 2011 Jun 16;474(7351):337- 42
- Wong WP, Tiano JP, Liu S, Hewitt SC, Le May C, Dalle S, Katzenellenbogen JA, Katzenellenbogen BS, Korach KS, Mauvais-Jarvis F, Extranuclear estrogen receptor-alpha stimulates NeuroD1 binding to the insulin promoter and favors insulin synthesis. Proc Natl Acad Sci U S A. 2010 Jul 20;107(29):13057-62
- Isensee J, Meoli L, Zazzu V, Nabzdyk C, Witt H, Soewarto D, Effertz K, Fuchs H, Gailus-Durner V, Busch D, Adler T, de Angelis MH, Irgang M, Otto C, Noppinger PR, Expression pattern of G protein-coupled receptor 30 in LacZ reporter mice. Endocrinology. 2009 Apr;150(4):1722-30

- Liu S, Le May C, Wong WP, Ward RD, Clegg DJ, Marcelli M, Korach KS, Mauvais-Jarvis F, Importance of extranuclear estrogen receptor-alpha and membrane G protein-coupled estrogen receptor in pancreatic islet survival. Diabetes. 2009 Oct;58(10):2292-302

- Haas E, Bhattacharya I, Brailoiu E, Damjanovic M, Brailoiu GC, Gao X, Mueller-Guerre L, Marjon NA, Gut A, Minotti R, Meyer MR, Amann K, Ammann E, Perez-Dominguez A, Genoni M, Clegg DJ, Dun NJ, Resta TC, Prossnitz ER, Barton M, Regulatory role of G protein-coupled estrogen receptor for vascular function and obesity. Circ Res. 2009 Feb 13;104(3):288-91

- Otto C, Fuchs I, Kauselmann G, Kern H, Zevnik B, Andreasen P, Schwarz G, Altmann H, Klewer M, Schoor M, Vonk R, Fritzemeier KH, GPR30 does not mediate estrogenic responses in reproductive organs in mice. Biol Reprod. 2009 Jan;80(1):34-41

- Wang C, Dehghani B, Li Y, Kaler LJ, Proctor T, Vandenbark AA, Offner H, Membrane estrogen receptor regulates experimental autoimmune encephalomyelitis through up-regulation of programmed death 1. J Immunol. 2009 Mar 1;182(5):3294-303

- Martensson UE, Salehi SA, Windahl S, Gomez MF, Sward K, Daszkiewicz-Nilsson J, Wendt A, Andersson N, Hellstrand P, Grande PO, Owman C, Rosen CJ, Adamo ML, Lundquist I, Rorsman P, Nilsson BO, Ohlsson C, Olde B, Leeb-Lundberg LM, Deletion of the G protein-coupled receptor 30 impairs glucose tolerance, reduces bone growth, increases blood pressure, and eliminates estradiol-stimulated insulin release in female mice. Endocrinology. 2009 Feb;150(2):687-98

- Windahl SH, Andersson N, Chagin AS, Martensson UE, Carlsten H, Olde B, Swanson C, Moverare-Skrtic S, Savendahl L, Lagerquist MK, Leeb-Lundberg LM, Ohlsson C, The role of the G protein-coupled receptor GPR30 in the effects of estrogen in ovariectomized mice. Am J Physiol Endocrinol Metab. 2009 Mar;296(3):E490-6

- Wang C, Dehghani B, Magrisso IJ, Rick EA, Bonhomme E, Cody DB, Elenich LA, Subramanian S, Murphy SJ, Kelly MJ, Rosenbaum JS, Vandenbark AA, Offner H, GPR30 Contributes to Estrogen-Induced Thymic Atrophy. Mol Endocrinol. 2008 Mar;22(3):636-48

GPR33

Knockout references

http://www.mousephenotype.org/data/genes/MGI:1277106;
http://www.informatics.jax.org/allele/MGI:4455795?page=alleleDetail&id=MGI:4455795

Phenotype references

- Lyon MF, Rastan S, Brown SDM, in Lyon MF, Rastan S, Brown SDM (eds), Genetic Variants and Strains of the Laboratory Mouse. 3rd Edition. 1996Oxford: Oxford University Press.

- Roderick TH, Using inversions to detect and study recessive lethals and detrimentals in mice

- de Serres FJ, Sheridan W (eds), Utilization of Mammalian Specific Locus Studies in Hazard Evaluation and Estimation of Genetic Risk. 1983:135-67. Plenum Publishing Corp..

GPR34

Knockout references

http://www.mousephenotype.org/data/genes/MGI:1346334
http://www.informatics.jax.org/marker/MGI:1346334

Phenotype references

- Preissler J, Grosche A, Lede V, Le Duc D, Krugel K, Matyash V, Szulzewsky F, Kallendrusch S, Immig K, Kettenmann H, Bechmann I, Schoneberg T, Schulz A, Altered microglial phagocytosis in GPR34-deficient mice. Glia. 2015 Feb;63(2):206-15

- Barnes MJ, Li CM, Xu Y, An J, Huang Y, Cyster JG, The lysophosphatidylserine receptor GPR174 constrains regulatory T cell development and function. J Exp Med. 2015 Jun 29;212(7):1011-20

- Liebscher I, Muller U, Teupser D, Engemaier E, Engel KM, Ritscher L, Thor D, Sangkuhl K, Ricken A, Wurm A, Piehler D, Schmutzler S, Fuhrmann H, Albert FW, Reichenbach A, Thiery J, Schoneberg T, Schulz A, Altered immune response in mice deficient for the G protein-coupled receptor GPR34. J Biol Chem. 2011 Jan 21;286(3):2101-10

GPR35

Knockout references

http://www.mousephenotype.org/data/genes/MGI:1929509;
http://www.informatics.jax.org/allele/MGI:5520229?page=alleleDetail&id=MGI:5520229

Phenotype references

- Skarnes WC, Rosen B, West AP, Koutsourakis M, Bushell W, Iyer V, Mujica AO, Thomas M, Harrow J, Cox T, Jackson D, Severin J, Biggs P, Fu J, Nefedov M, de Jong PJ, Stewart AF, Bradley A, A conditional knockout resource for the genome-wide study of mouse gene function. Nature. 2011 Jun 16;474(7351):337- 42

- Lyon MF, Rastan S, Brown SDM, in Lyon MF, Rastan S, Brown SDM (eds), Genetic Variants and Strains of the Laboratory Mouse. 3rd Edition. 1996Oxford: Oxford University Press.

- Juriloff DM, Sulik KK, Roderick TH, Hogan BK, Genetic and developmental studies of a new mouse mutation that produces otocephaly. J Craniofac Genet Dev Biol. 1985;5(2):121-45

- Roderick TH, Using inversions to detect and study recessive lethals and detrimentals in mice, in de Serres FJ, Sheridan W (eds), Utilization of Mammalian Specific Locus Studies in Hazard Evaluation and Estimation of Genetic Risk. 1983:135-67. Plenum Publishing Corp..

- Roderick TH, Hawes NL, Two radiation-induced chromosomal inversions in mice (Mus musculus). Proc Natl Acad Sci U S A. 1970 Oct;67(2):961-7

GPR37

Knockout references

http://www.mousephenotype.org/data/genes/MGI:1313297
http://www.informatics.jax.org/marker/MGI:1313297

Phenotype references

- La Sala G, Marazziti D, Di Pietro C, Golini E, Matteoni R, Tocchini-Valentini GP, Modulation of Dhh signaling and altered Sertoli cell function in mice lacking the GPR37-prosaposin receptor. FASEB J. 2015 May;29(5):2059-69

- Gandia J, Morato X, Stagljar I, Fernandez-Duenas V, Ciruela F, Adenosine A2A receptor-mediated control of pilocarpine-induced tremulous jaw movements is Parkinson's disease-associated GPR37 receptor- dependent. Behav Brain Res. 2015 Jul 15;288:103-6

- Mandillo S, Golini E, Marazziti D, Di Pietro C, Matteoni R, Tocchini-Valentini GP, Mice lacking the Parkinson's related GPR37/PAEL receptor show non-motor behavioral phenotypes: age and gender effect. Genes Brain Behav. 2013 Jun;12(4):465-77

- Marazziti D, Di Pietro C, Mandillo S, Golini E, Matteoni R, Tocchini-Valentini GP, Absence of the GPR37/PAEL receptor impairs striatal Akt and ERK2 phosphorylation, {Delta}FosB expression, and conditioned place preference to amphetamine and cocaine. FASEB J. 2011 Jun;25(6):2071-81

- Marazziti D, Di Pietro C, Golini E, Mandillo S, Matteoni R, Tocchini-Valentini GP, Induction of macroautophagy by overexpression of the Parkinson's disease-associated GPR37 receptor. FASEB J. 2009 Jun;23(6):1978-87

- Roesch K, Jadhav AP, Trimarchi JM, Stadler MB, Roska B, Sun BB, Cepko CL, The transcriptome of retinal Muller glial cells. J Comp Neurol. 2008 Jul 10;509(2):225-38

- Wang HQ, Imai Y, Inoue H, Kataoka A, Iita S, Nukina N, Takahashi R, Pael-R transgenic mice crossed with parkin deficient mice displayed progressive and selective catecholaminergic neuronal loss. J Neurochem. 2008 Oct;107(1):171-85

- Imai Y, Inoue H, Kataoka A, Hua-Qin W, Masuda M, Ikeda T, Tsukita K, Soda M, Kodama T, Fuwa T, Honda Y, Kaneko S, Matsumoto S, Wakamatsu K, Ito S, Miura M, Aosaki T, Itohara S, Takahashi R, Pael receptor is involved in dopamine metabolism in the nigrostriatal system. Neurosci Res. 2007 Dec;59(4):413-25

- Marazziti D, Mandillo S, Di Pietro C, Golini E, Matteoni R, Tocchini-Valentini GP, GPR37 associates with the dopamine transporter to modulate dopamine uptake and behavioral responses to dopaminergic drugs. Proc Natl Acad Sci U S A. 2007 Jun 5;104(23):9846-51

- Marazziti D, Golini E, Mandillo S, Magrelli A, Witke W, Matteoni R, Tocchini-Valentini GP, Altered dopamine signaling and MPTP resistance in mice lacking the Parkinson's disease-associated GPR37/parkin-associated endothelin-like receptor. Proc Natl Acad Sci U S A. 2004 Jul 6;101(27):10189

GPR37l1

Knockout references

http://www.mousephenotype.org/data/genes/MGI:1928503
http://www.informatics.jax.org/marker/MGI:1928503

Phenotype references

- Marazziti D, Di Pietro C, Golini E, Mandillo S, La Sala G, Matteoni R, Tocchini-Valentini GP, Precocious cerebellum development and improved motor functions in mice lacking the astrocyte cilium-, patched 1-associated Gpr37l1 receptor. Proc Natl Acad Sci U S A. 2013 Oct 8;110(41):16486-16491

- Skarnes WC, Rosen B, West AP, Koutsourakis M, Bushell W, Iyer V, Mujica AO, Thomas M, Harrow J, Cox T, Jackson D, Severin J, Biggs P, Fu J, Nefedov M, de Jong PJ, Stewart AF, Bradley A, A conditional knockout resource for the genome-wide study of mouse gene function. Nature. 2011 Jun 16;474(7351):337- 42

- Zambrowicz BP, Abuin A, Ramirez-Solis R, Richter LJ, Piggott J, BeltrandelRio H, Buxton EC, Edwards J, Finch RA, Friddle CJ, Gupta A, Hansen G, Hu Y, Huang W, Jaing C, Key BW Jr, Kipp P, Kohlhauff B, Ma ZQ, Markesich D, Payne R, Potter DG, Qian N, Shaw J, Schrick J, Shi ZZ, Sparks MJ, Van Sligtenhorst I, Vogel P, Walke W, Xu N, Zhu Q, Person C, Sands AT, Wnk1 kinase deficiency lowers blood pressure in mice: a gene-trap screen to identify potential targets for therapeutic intervention. Proc Natl Acad Sci U S A. 2003 Nov 25;100(24):14109-14

- Lyon MF, Rastan S, Brown SDM, in Lyon MF, Rastan S, Brown SDM (eds), Genetic Variants and Strains of the Laboratory Mouse. 3rd Edition. 1996Oxford: Oxford University Press.

- Juriloff DM, Sulik KK, Roderick TH, Hogan BK, Genetic and developmental studies of a new mouse mutation that produces otocephaly. J Craniofac Genet Dev Biol. 1985;5(2):121-45

- Roderick TH, Using inversions to detect and study recessive lethals and detrimentals in mice, in de Serres FJ, Sheridan W (eds), Utilization of Mammalian Specific Locus Studies in Hazard Evaluation and Estimation of Genetic Risk. 1983:135-67. Plenum Publishing Corp..

- Roderick TH, Hawes NL, Two radiation-induced chromosomal inversions in mice (Mus musculus). Proc Natl Acad Sci U S A. 1970 Oct;67(2):961-7

GPR39

Knockout references

http://www.mousephenotype.org/data/genes/MGI:1918361
http://www.informatics.jax.org/marker/MGI:1918361

Phenotype references

- Mlyniec K, Gawel M, Nowak G, Study of antidepressant drugs in GPR39 (zinc receptor(-)/(-)) knockout mice, showing no effect of conventional antidepressants, but effectiveness of NMDA antagonists. Behav Brain Res. 2015;287:135-8

- Zeng F, Wind N, McClenaghan C, Verkuyl JM, Watson RP, Nash MS, GPR39 is coupled to TMEM16A in intestinal fibroblast-like cells. PLoS One. 2012;7(10):e47686

- Petersen PS, Jin C, Madsen AN, Rasmussen M, Kuhre R, Egerod KL, Nielsen LB, Schwartz TW, Holst B, Deficiency of the GPR39 receptor is associated with obesity and altered adipocyte metabolism. FASEB J. 2011 Nov;25(11):3803-14

- Tremblay F, Richard AM, Will S, Syed J, Stedman N, Perreault M, Gimeno RE, Disruption of G protein- coupled receptor 39 impairs insulin secretion in vivo. Endocrinology. 2009 Jun;150(6):2586-95

- Holst B, Egerod KL, Jin C, Petersen PS, Ostergaard MV, Hald J, Sprinkel AM, Storling J, Mandrup-Poulsen T, Holst JJ, Thams P, Orskov C, Wierup N, Sundler F, Madsen OD, Schwartz TW, G protein-coupled receptor 39 deficiency is associated with pancreatic islet dysfunction. Endocrinology. 2009 Jun;150(6):2577-85

- Tremblay F, Perreault M, Klaman LD, Tobin JF, Smith E, Gimeno RE, Normal food intake and body weight in mice lacking the G protein-coupled receptor GPR39. Endocrinology. 2007 Feb;148(2):501-6

- Moechars D, Depoortere I, Moreaux B, de Smet B, Goris I, Hoskens L, Daneels G, Kass S, Ver Donck L, Peeters T, Coulie B, Altered gastrointestinal and metabolic function in the GPR39-obestatin receptor- knockout mouse. Gastroenterology. 2006 Oct;131(4):1131-41

- Lyon MF, Rastan S, Brown SDM, in Lyon MF, Rastan S, Brown SDM (eds), Genetic Variants and Strains of the Laboratory Mouse. 3rd Edition. 1996Oxford: Oxford University Press.

- Juriloff DM, Sulik KK, Roderick TH, Hogan BK, Genetic and developmental studies of a new mouse mutation that produces otocephaly. J Craniofac Genet Dev Biol. 1985;5(2):121-45

- Roderick TH, Using inversions to detect and study recessive lethals and detrimentals in mice, in de Serres FJ, Sheridan W (eds), Utilization of Mammalian Specific Locus Studies in Hazard Evaluation and Estimation of Genetic Risk. 1983:135-67. Plenum Publishing Corp..

- Roderick TH, Hawes NL, Two radiation-induced chromosomal inversions in mice (Mus musculus). Proc Natl Acad Sci U S A. 1970 Oct;67(2):961-7

GPR40

Knockout references

http://www.mousephenotype.org/data/genes/MGI:2684079
http://www.informatics.jax.org/marker/MGI:2684079

Phenotype references

- Wauquier F, Philippe C, Leotoing L, Mercier S, Davicco MJ, Lebecque P, Guicheux J, Pilet P, Miot-Noirault E, Poitout V, Alquier T, Coxam V, Wittrant Y, The free fatty acid receptor G protein-coupled receptor 40 (GPR40) protects from bone loss through inhibition of osteoclast differentiation. J Biol Chem. 2013 Mar 1;288(9):6542-51

- Honore JC, Kooli A, Hamel D, Alquier T, Rivera JC, Quiniou C, Hou X, Kermorvant-Duchemin E, Hardy P, Poitout V, Chemtob S, Fatty acid receptor Gpr40 mediates neuromicrovascular degeneration induced by transarachidonic acids in rodents. Arterioscler Thromb Vasc Biol. 2013 May;33(5):954-61

- Xiong Y, Swaminath G, Cao Q, Yang L, Guo Q, Salomonis H, Lu J, Houze JB, Dransfield PJ, Wang Y, Liu JJ, Wong S, Schwandner R, Steger F, Bar bault H, Liu L, Coberly S, Miao L, Zhang J, Lin DC, Schwarz M, Activation of FFA1 mediates GLP-1 secretion in mice. Evidence for allosterism at FFA1. Mol Cell Endocrinol. 2013 Apr 30;369(1-2):119-29

- Freigang S, Ampenberger F, Weiss A, Kanneganti TD, Iwakura Y, Hersberger M, Kopf M, Fatty acid-induced mitochondrial uncoupling elicits inflammasome-independent IL-1alpha and sterile vascular inflammation in atherosclerosis. Nat Immunol. 2013 Oct;14(10):1045-53

- Godinot N, Yasumatsu K, Barcos ME, Pineau N, Ledda M, Viton F, Ninomiya Y, le Coutre J, Damak S, Activation of tongue-expressed GPR40 and GPR120 by non caloric agonists is not sufficient to drive preference in mice. Neuroscience. 2013 Oct 10;250:20-30

- Sclafani A, Zukerman S, Ackroff K, GPR40 and GPR120 fatty acid sensors are critical for postoral but not oral mediation of fat preferences in the mouse. Am J Physiol Regul Integr Comp Physiol. 2013 Dec 15;305(12):R1490-7

- Ferdaoussi M, Bergeron V, Zarrouki B, Kolic J, Cantley J, Fielitz J, Olson EN, Prentki M, Biden T, MacDonald PE, Poitout V, G protein-coupled receptor (GPR)40-dependent potentiation of insulin secretion in mouse islets is mediated by protein kinase D1. Diabetologia. 2012 Oct;55(10):2682-92

- Cartoni C, Yasumatsu K, Ohkuri T, Shigemura N, Yoshida R, Godinot N, le Coutre J, Ninomiya Y, Damak S, Taste preference for fatty acids is mediated by GPR40 and GPR120. J Neurosci. 2010 Jun 23;30(25):8376- 82

GPR41

Knockout references

http://www.mousephenotype.org/data/genes/MGI:2685324
http://www.informatics.jax.org/marker/MGI:2685324

Phenotype references

- Pluznick JL, Protzko RJ, Gevorgyan H, Peterlin Z, Sipos A, Han J, Brunet I, Wan LX, Rey F, Wang T, Firestein SJ, Yanagisawa M, Gordon JI, Eichmann A, Peti-Peterdi J, Caplan MJ, Olfactory receptor responding to gut microbiota-derived signals plays a role in renin secretion and blood pressure regulation. Proc Natl Acad Sci U S A. 2013 Mar 12;110(11):4410-5

- Inoue D, Kimura I, Wakabayashi M, Tsumoto H, Ozawa K, Hara T, Takei Y, Hirasawa A, Ishihama Y, Tsujimoto G, Short-chain fatty acid receptor GPR41-mediated activation of sympathetic neurons involves synapsin 2b phosphorylation. FEBS Lett. 2012 May 21;586(10):1547-54

- Tolhurst G, Heffron H, Lam YS, Parker HE, Hab b AM, Diakogiannaki E, Cameron J, Grosse J, Reimann F, Gribble FM, Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein-coupled receptor FFAR2. Diabetes. 2012 Feb;61(2):364-71

- Lin HV, Frassetto A, Kowalik EJ Jr, Nawrocki AR, Lu MM, Kosinski JR, Hubert JA, Szeto D, Yao X, Forrest G, Marsh DJ, Butyrate and propionate protect against diet-induced obesity and regulate gut hormones via free fatty acid receptor 3-independent mechanisms. PLoS One. 2012;7(4):e35240

- Kimura I, Inoue D, Maeda T, Hara T, Ichimura A, Miyauchi S, Kobayashi M, Hirasawa A, Tsujimoto G, Short-chain fatty acids and ketones directly regulate sympathetic nervous system via G protein-coupled receptor 41 (GPR41). Proc Natl Acad Sci U S A. 2011 May 10;108(19):8030-5

- Zaibi MS, Stocker CJ, O'Dowd J, Davies A, Bellahcene M, Cawthorne MA, Brown AJ, Smith DM, Arch JR, Roles of GPR41 and GPR43 in leptin secretory responses of murine adipocytes to short chain fatty acids. FEBS Lett. 2010 Jun 3;584(11):2381-6

- Helmholtz Zentrum Muenchen GmbH, Alleles produced for the EUCOMM and EUCOMMTools projects by the Helmholtz Zentrum Muenchen GmbH (Hmgu). MGI Direct Data Submission. 2010-2015;

- Samuel BS, Shaito A, Motoike T, Rey FE, Backhed F, Manchester JK, Hammer RE, Williams SC, Crowley J, Yanagisawa M, Gordon JI, Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41. Proc Natl Acad Sci U S A. 2008 Oct 28;105(43):16767-72

GPR43

Knockout references

http://www.mousephenotype.org/data/genes/MGI:2441731
http://www.informatics.jax.org/marker/MGI:2685324

Phenotype references

- Macia L, Tan J, Vieira AT, Leach K, Stanley D, Luong S, Maruya M, Ian McKenzie C, Hij kata A, Wong C, Binge L, Thorburn AN, Chevalier N, Ang C, Marino E, Robert R, Offermanns S, Teixeira MM, Moore RJ, Flavell RA, Fagarasan S, Mackay CR, Metabolite-sensing receptors GPR43 and GPR109A facilitate dietary fibre-induced gut homeostasis through regulation of the inflammasome. Nat Commun. 2015;6:6734

- Kim MH, Kang SG, Park JH, Yanagisawa M, Kim CH, Short-chain fatty acids activate GPR41 and GPR43 on intestinal epithelial cells to promote inflammatory responses in mice. Gastroenterology. 2013 Aug;145(2):396-406.e1-10

- Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, Bohlooly-Y M, Glickman JN, Garrett WS, The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. Science. 2013 Aug 2;341(6145):569-73

- Tolhurst G, Heffron H, Lam YS, Parker HE, Hab b AM, Diakogiannaki E, Cameron J, Grosse J, Reimann F, Gribble FM, Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein-coupled receptor FFAR2. Diabetes. 2012 Feb;61(2):364-71

- Bjursell M, Admyre T, Goransson M, Marley AE, Smith DM, Oscarsson J, Bohlooly-Y M, Improved glucose control and reduced body fat mass in free fatty acid receptor 2-deficient mice fed a high-fat diet. Am J Physiol Endocrinol Metab. 2011 Jan;300(1):E211-20

- Kimura I, Inoue D, Maeda T, Hara T, Ichimura A, Miyauchi S, Kobayashi M, Hirasawa A, Tsujimoto G, Short-chain fatty acids and ketones directly regulate sympathetic nervous system via G protein-coupled receptor 41 (GPR41). Proc Natl Acad Sci U S A. 2011 May 10;108(19):8030-5

- Vinolo MA, Ferguson GJ, Kulkarni S, Damoulakis G, Anderson K, Bohlooly-Y M, Stephens L, Hawkins PT, Curi R, SCFAs induce mouse neutrophil chemotaxis through the GPR43 receptor. PLoS One. 2011;6(6):e21205

- Maslowski KM, Vieira AT, Ng A, Kranich J, Sierro F, Yu D, Schilter HC, Rolph MS, Mackay F, Artis D, Xavier RJ, Teixeira MM, Mackay CR, Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. Nature. 2009 Oct 29;461(7268):1282-6

- Sina C, Gavrilova O, Forster M, Till A, Derer S, Hildebrand F, Raabe B, Chalaris A, Scheller J, Rehmann A, Franke A, Ott S, Hasler R, Nikolaus S, Folsch UR, Rose-John S, Jiang HP, Li J, Schreiber S, Rosenstiel P, G protein-coupled receptor 43 is essential for neutrophil recruitment during intestinal inflammation. J Immunol. 2009 Dec 1;183(11):7514-22

- Ge H, Li X, Weiszmann J, Wang P, Baribault H, Chen JL, Tian H, Li Y, Activation of g protein-coupled receptor 43 in adipocytes leads to inh bition of lipolysis and suppression of plasma free Fatty acids. Endocrinology. 2008 Sep;149(9):4519-26

GPR44

Knockout references

http://www.mousephenotype.org/data/genes/MGI:1330275
http://www.informatics.jax.org/marker/MGI:1330275

Phenotype references

- Trimarco A, Forese MG, Alfieri V, Lucente A, Brambilla P, Dina G, Pieragostino D, Sacchetta P, Urade Y, Boizet-Bonhoure B, Martinelli Boneschi F, Quattrini A, Taveggia C, Prostaglandin D2 synthase/GPR44: a signaling axis in PNS myelination. Nat Neurosci. 2014 Dec;17(12):1682-92

- Kaushik MK, Aritake K, Kamauchi S, Hayaishi O, Huang ZL, Lazarus M, Urade Y, Prostaglandin D(2) is crucial for seizure suppression and postictal sleep. Exp Neurol. 2014 Mar;253:82-90

- Moniot B, Ujjan S, Champagne J, Hirai H, Aritake K, Nagata K, Dubois E, Nidelet S, Nakamura M, Urade Y, Poulat F, Boizet-Bonhoure B, Prostaglandin D2 acts through the Dp2 receptor to influence male germ cell differentiation in the foetal mouse testis. Development. 2014 Sep;141(18):3561-71

- Haba R, Shintani N, Onaka Y, Kanoh T, Wang H, Takenaga R, Hayata A, Hirai H, Nagata KY, Nakamura M, Kasai A, Hashimoto R, Nagayasu K, Nakazawa T, Hashimoto H, Baba A, Central CRTH2, a second prostaglandin D2 receptor, mediates emotional impairment in the lipopolysaccharide and tumor-induced sickness behavior model. J Neurosci. 2014 Feb 12;34(7):2514-23

- Sarashina H, Tsubosaka Y, Omori K, Aritake K, Nakagawa T, Hori M, Hirai H, Nakamura M, Narumiya S, Urade Y, Ozaki H, Murata T, Opposing immunomodulatory roles of prostaglandin D2 during the progression of skin inflammation. J Immunol. 2014 Jan 1;192(1):459-65Nelson AM, Loy DE, Lawson JA, Katseff AS, Fitzgerald GA, Garza LA, Prostaglandin D2 inhibits wound-induced hair follicle neogenesis through the receptor, Gpr44. J Invest Dermatol. 2013 Apr;133(4):881-9

GPR48

Knockout references

http://www.mousephenotype.org/data/genes/MGI:1891468
http://www.informatics.jax.org/marker/MGI:1891468

Phenotype references

- Pan H, Cui H, Liu S, Qian Y, Wu H, Li L, Guan Y, Guan X, Zhang L, Fan HY, Ma Y, Li R, Liu M, Li D, Lgr4 gene regulates corpus luteum maturation through modulation of the WNT-mediated EGFR-ERK signaling pathway. Endocrinology. 2014 Sep;155(9):3624-37

- Kinzel B, Pikiolek M, Orsini V, Sprunger J, Isken A, Zietzling S, Desplanches M, Dubost V, Breustedt D, Valdez R, Liu D, Theil D, Muller M, Dietrich B, Bouwmeester T, Ruffner H, Tchorz JS, Functional roles of Lgr4 and Lgr5 in embryonic gut, kidney and skin development in mice. Dev Biol. 2014 Jun 15;390(2):181-90

- Yi T, Weng J, Siwko S, Luo J, Li D, Liu M, LGR4/GPR48 inactivation leads to aniridia-genitourinary anomalies-mental retardation syndrome defects. J Biol Chem. 2014 Mar 28;289(13):8767-80

- Dang Y, Liu B, Xu P, Zhu P, Zhai Y, Liu M, Ye X, Gpr48 deficiency induces polycystic kidney lesions and renal f brosis in mice by activating Wnt signal pathway. PLoS One. 2014;9(3):e89835

- Wang F, Zhang X, Wang J, Chen M, Fan N, Ma Q, Liu R, Wang R, Li X, Liu M, Ning G, LGR4 acts as a link between the peripheral circadian clock and lipid metabolism in liver. J Mol Endocrinol. 2014 Apr;52(2):133-43

- Qian Y, Liu S, Guan Y, Pan H, Guan X, Qiu Z, Li L, Gao N, Zhao Y, Li X, Lu Y, Liu M, Li D, Lgr4-mediated Wnt/beta-catenin signaling in peritubular myoid cells is essential for spermatogenesis. Development. 2013 Apr;140(8):1751-61

- Liu S, Qian Y, Li L, Wei G, Guan Y, Pan H, Guan X, Zhang L, Lu X, Zhao Y, Liu M, Li D, Lgr4 gene deficiency increases suscept bility and severity of dextran sodium sulfate-induced inflammatory bowel disease in mice. J Biol Chem. 2013 Mar 29;288(13):8794-803; discussion 8804

- Du B, Luo W, Li R, Tan B, Han H, Lu X, Li D, Qian M, Zhang D, Zhao Y, Liu M, Lgr4/Gpr48 negatively regulates TLR2/4-associated pattern recognition and innate immunity by targeting CD14 expression. J Biol Chem. 2013 May 24;288(21):15131-41

- Sone M, Oyama K, Mohri Y, Hayashi R, Clevers H, Nishimori K, LGR4 expressed in uterine epithelium is necessary for uterine gland development and contributes to decidualization in mice. FASEB J. 2013 Dec;27(12):4917-28

- Luo W, Rodriguez M, Valdez JM, Zhu X, Tan K, Li D, Siwko S, Xin L, Liu M, Lgr4 is a key regulator of prostate development and prostate stem cell differentiation. Stem Cells. 2013 Nov;31(11):2492-505

- Wang J, Liu R, Wang F, Hong J, Li X, Chen M, Ke Y, Zhang X, Ma Q, Wang R, Shi J, Cui B, Gu W, Zhang Y, Zhang Z, Wang W, Xia X, Liu M, Ning G, Ablation of LGR4 promotes energy expenditure by driving white-to-brown fat switch. Nat Cell Biol. 2013 Dec;15(12):1455-63

- Wang Y, Dong J, Li D, Lai L, Siwko S, Li Y, Liu M, Lgr4 regulates mammary gland development and stem cell activity through the pluripotency transcription factor Sox2. Stem Cells. 2013 Sep;31(9):1921-31

- Mustata RC, Vasile G, Fernandez-Vallone V, Strollo S, Lefort A, Libert F, Monteyne D, Perez-Morga D, Vassart G, Garcia MI, Identification of Lgr5-independent spheroid-generating progenitors of the mouse fetal intestinal epithelium. Cell Rep. 2013 Oct 31;5(2):421-32

- Mohri Y, Oyama K, Sone M, Akamatsu A, Nishimori K, LGR4 Is Required for the Cell Survival of the Peripheral Mesenchyme at the Embryonic Stages of Nephrogenesis. Biosci Biotechnol Biochem. 2012 May 23;76(5):888-91

- Mohri Y, Oyama K, Akamatsu A, Kato S, Nishimori K, Lgr4-deficient mice showed premature differentiation of ureteric bud with reduced expression of Wnt effector Lef1 and Gata3. Dev Dyn. 2011 Jun;240(6):1626-34

- de Lau W, Barker N, Low TY, Koo BK, Li VS, Teunissen H, Kujala P, Haegebarth A, Peters PJ, van de Wetering M, Stange DE, van Es JE, Guardavaccaro D, Schasfoort RB, Mohri Y, Nishimori K, Mohammed S, Heck AJ, Clevers H, Lgr5 homologues associate with Wnt receptors and mediate R-spondin signalling. Nature. 2011 Aug 18;476(7360):293-7

- Mustata RC, Van Loy T, Lefort A, L bert F, Strollo S, Vassart G, Garcia MI, Lgr4 is required for Paneth cell differentiation and maintenance of intestinal stem cells ex vivo. EMBO Rep. 2011 Jun;12(6):558-64

- Wang Z, Jin C, Li H, Li C, Hou Q, Liu M, Dong Xda E, Tu L, GPR48-Induced keratinocyte proliferation occurs through HB-EGF mediated EGFR transactivation. FEBS Lett. 2010 Sep 24;584(18):4057-62

- Li XY, Lu Y, Sun HY, Wang JQ, Yang J, Zhang HJ, Fan NG, Xu J, Jiang JJ, Liu RY, Li DL, Liu MY, Ning G, G protein-coupled receptor 48 upregulates estrogen receptor alpha expression via cAMP/PKA signaling in the male reproductive tract. Development. 2010 Jan;137(1):151-7

- Snippert HJ, Haegebarth A, Kasper M, Jaks V, van Es JH, Barker N, van de Wetering M, van den Born M, Begthel H, Vries RG, Stange DE, Toftgard R, Clevers H, Lgr6 marks stem cells in the hair follicle that generate all cell lineages of the skin. Science. 2010 Mar 12;327(5971):1385-9

- Favor J, Bradley A, Conte N, Janik D, Pretsch W, Reitmeir P, Rosemann M, Schmahl W, Wienberg J, Zaus I, Analysis of Pax6 Contiguous Gene Deletions in the Mouse, Mus musculus, Identifies Regions Distinct from Pax6 Responsible for Extreme Small-Eye and Belly-Spotting Phenotypes. Genetics. 2009 Aug;182(4):1077-88

- Luo J, Zhou W, Zhou X, Li D, Weng J, Yi Z, Cho SG, Li C, Yi T, Wu X, Li XY, de Crombrugghe B, Hook M, Liu M, Regulation of bone formation and remodeling by G-protein-coupled receptor 48. Development. 2009 Aug;136(16):2747-56

- Lambot MA, Mendive F, Laurent P, Van Schoore G, Noel JC, Vanderhaeghen P, Vassart G, Three- dimensional reconstruction of efferent ducts in wild-type and Lgr4 knock-out mice. Anat Rec (Hoboken). 2009 Apr;292(4):595-603

- Yamashita R, Takegawa Y, Sakumoto M, Nakahara M, Kawazu H, Hoshii T, Araki K, Yokouchi Y, Yamamura K, Defective development of the gall bladder and cystic duct in Lgr4- hypomorphic mice. Dev Dyn. 2009 Apr;238(4):993-1000

- Weng J, Luo J, Cheng X, Jin C, Zhou X, Qu J, Tu L, Ai D, Li D, Wang J, Martin JF, Amendt BA, Liu M, Deletion of G protein-coupled receptor 48 leads to ocular anterior segment dysgenesis (ASD) through down- regulation of Pitx2. Proc Natl Acad Sci U S A. 2008 Apr 22;105(16):6081-6

- Mohri Y, Kato S, Umezawa A, Okuyama R, Nishimori K, Impaired hair placode formation with reduced expression of hair follicle-related genes in mice lacking Lgr4. Dev Dyn. 2008 Aug;237(8):2235-42

- Jin C, Yin F, Lin M, Li H, Wang Z, Weng J, Liu M, Da Dong X, Qu J, Tu L, GPR48 regulates epithelial cell proliferation and migration by activating EGFR during eyelid development. Invest Ophthalmol Vis Sci. 2008 Oct;49(10):4245-53

- Hansen GM, Markesich DC, Burnett MB, Zhu Q, Dionne KM, Richter LJ, Finnell RH, Sands AT, Zambrowicz BP, Abuin A, Large-scale gene trapping in C57BL/6N mouse embryonic stem cells. Genome Res. 2008 Oct;18(10):1670-9

- Song H, Luo J, Luo W, Weng J, Wang Z, Li B, Li D, Liu M, Inactivation of G-protein-coupled Receptor 48 (Gpr48/Lgr4) Impairs Definitive Erythropoiesis at Midgestation through Down-regulation of the ATF4 Signaling Pathway. J Biol Chem. 2008 Dec 26;283(52):36687-97

- Hoshii T, Takeo T, Nakagata N, Takeya M, Araki K, Yamamura K, LGR4 Regulates the Postnatal Development and Integrity of Male Reproductive Tracts in Mice. Biol Reprod. 2007 Feb;76(2):303-13

- Kato S, Mohri Y, Matsuo T, Ogawa E, Umezawa A, Okuyama R, Nishimori K, Eye-open at birth phenotype with reduced keratinocyte motility in LGR4 null mice. FEBS Lett. 2007 Oct 2;581(24):4685-90

- Friedel RH, Seisenberger C, Kaloff C, Wurst W, EUCOMM--the European conditional mouse mutagenesis program. Brief Funct Genomic Proteomic. 2007 Sep;6(3):180-5

- Mendive F, Laurent P, Van Schoore G, Skarnes W, Pochet R, Vassart G, Defective postnatal development of the male reproductive tract in LGR4 knockout mice. Dev Biol. 2006 Feb 15;290(2):421-34

- Kato S, Matsubara M, Matsuo T, Mohri Y, Kazama I, Hatano R, Umezawa A, Nishimori K, Leucine-rich repeat-containing G protein-coupled receptor-4 (LGR4, Gpr48) is essential for renal development in mice. Nephron Exp Nephrol. 2006;104(2):e63-75

- Taniwaki T, Haruna K, Nakamura H, Sekimoto T, Oike Y, Imaizumi T, Saito F, Muta M, Soejima Y, Utoh A, Nakagata N, Araki M, Yamamura K, Araki K, Characterization of an exchangeable gene trap using pU-17 carrying a stop codon-beta geo cassette. Dev Growth Differ. 2005 Apr;47(3):163-72

- Van Schoore G, Mendive F, Pochet R, Vassart G, Expression pattern of the orphan receptor LGR4/GPR48 gene in the mouse. Histochem Cell Biol. 2005 Jul;124(1):35-50

- Mazerbourg S, Bouley DM, Sudo S, Klein CA, Zhang JV, Kawamura K, Goodrich LV, Rayburn H, Tessier-Lavigne M, Hsueh AJ, Leucine-rich repeat-containing, g protein-coupled receptor 4 null mice exhibit intrauterine growth retardation associated with embryonic and perinatal lethality. Mol Endocrinol. 2004 Sep;18(9):2241-54

- Stryke D, Kawamoto M, Huang CC, Johns SJ, King LA, Harper CA, Meng EC, Lee RE, Yee A, L'Italien L, Chuang PT, Young SG, Skarnes WC, Babbitt PC, Ferrin TE, BayGenomics: a resource of insertional mutations in mouse embryonic stem cells. Nucleic Acids Res. 2003 Jan 1;31(1):278-81

- Hansen J, Floss T, Van Sloun P, Fuchtbauer EM, Vauti F, Arnold HH, Schnutgen F, Wurst W, von Melchner H, Ruiz P, A large-scale, gene-driven mutagenesis approach for the functional analysis of the mouse genome. Proc Natl Acad Sci U S A. 2003 Aug 19;100(17):9918-22

- Zambrowicz BP, Abuin A, Ramirez-Solis R, Richter LJ, Piggott J, BeltrandelRio H, Buxton EC, Edwards J, Finch RA, Friddle CJ, Gupta A, Hansen G, Hu Y, Huang W, Jaing C, Key BW Jr, Kipp P, Kohlhauff B, Ma ZQ, Markesich D, Payne R, Potter DG, Qian N, Shaw J, Schrick J, Shi ZZ, Sparks MJ, Van Sligtenhorst I, Vogel P, Walke W, Xu N, Zhu Q, Person C, Sands AT, Wnk1 kinase deficiency lowers blood pressure in mice: a gene-trap screen to identify potential targets for therapeutic intervention. Proc Natl Acad Sci U S A. 2003 Nov 25;100(24):14109-14

- Leighton PA, Mitchell KJ, Goodrich LV, Lu X, Pinson K, Scherz P, Skarnes WC, Tessier-Lavigne M, Defining brain wiring patterns and mechanisms through gene trapping in mice. Nature. 2001 Mar 8;410(6825):174-9

- Araki K, Imaizumi T, Sekimoto T, Yoshinobu K, Yoshimuta J, Akizuki M, Miura K, Araki M, Yamamura K, Exchangeable gene trap using the Cre/mutated lox system. Cell Mol Biol (Noisy-le-grand). 1999 Jul;45(5):737-50

- Lyon MF, Rastan S, Brown SDM, in Lyon MF, Rastan S, Brown SDM (eds), Genetic Variants and Strains of the Laboratory Mouse. 3rd Edition. 1996Oxford: Oxford University Press.

- Roderick TH, Using inversions to detect and study recessive lethals and detrimentals in mice, in de Serres FJ, Sheridan W (eds), Utilization of Mammalian Specific Locus Studies in Hazard Evaluation and Estimation of Genetic Risk. 1983:135-67. Plenum Publishing Corp..

- Roderick TH, Hawes NL, Nineteen paracentric chromosomal inversions in mice. Genetics. 1974 Jan;76(1):109-17

GPR49

Knockout references

http://www.mousephenotype.org/data/genes/MGI:1341817
http://www.informatics.jax.org/marker/MGI:1341817

Phenotype references

- Boquoi A, Arora S, Chen T, Litwin S, Koh J, Enders GH, Reversible cell cycle inhibition and premature aging features imposed by conditional expression of p16Ink4a. Aging Cell. 2015 Feb;14(1):139-47

- Horvay K, Jarde T, Casagranda F, Perreau VM, Haigh K, Nefzger CM, Akhtar R, Gridley T, Berx G, Haigh JJ, Barker N, Polo JM, Hime GR, Abud HE, Snai1 regulates cell lineage allocation and stem cell maintenance in the mouse intestinal epithelium. EMBO J. 2015 May 12;34(10):1319-35

- Li W, Wu J, Yang J, Sun S, Chai R, Chen ZY, Li H, Notch inh bition induces mitotically generated hair cells in mammalian cochleae via activating the Wnt pathway. Proc Natl Acad Sci U S A. 2015 Jan 6;112(1):166-71

- Peregrina K, Houston M, Daroqui C, Dhima E, Sellers RS, Augenlicht LH, Vitamin D is a determinant of mouse intestinal Lgr5 stem cell functions. Carcinogenesis. 2015 Jan;36(1):25-31

- Tao S, Tang D, Morita Y, Sperka T, Omrani O, Lechel A, Sakk V, Kraus J, Kestler HA, Kuhl M, Rudolph KL, Wnt activity and basal niche position sensitize intestinal stem and progenitor cells to DNA damage. EMBO J. 2015 Mar 4;34(5):624-40

- Lindley LE, Curtis KM, Sanchez-Mejias A, Rieger ME, Robbins DJ, Briegel KJ, The WNT-controlled transcriptional regulator LBH is required for mammary stem cell expansion and maintenance of the basal lineage. Development. 2015 Mar 1;142(5):893-904

- Wang S, Li N, Yousefi M, Nakauka-Ddamba A, Li F, Parada K, Rao S, Minuesa G, Katz Y, Gregory BD, Kharas MG, Yu Z, Lengner CJ, Transformation of the intestinal epithelium by the MSI2 RNA-binding protein. Nat Commun. 2015;6:6517

- Sakamaki A, Katsuragi Y, Otsuka K, Tomita M, Obata M, Iwasaki T, Abe M, Sato T, Ochiai M, Sakuraba Y, Aoyagi Y, Gondo Y, Sakimura K, Nakagama H, Mishima Y, Kominami R, Bcl11b SWI/SNF-complex subunit modulates intestinal adenoma and regeneration after gamma-irradiation through Wnt/beta-catenin pathway. Carcinogenesis. 2015 Jun;36(6):622-31

- Schuijers J, Junker JP, Mokry M, Hatzis P, Koo BK, Sasselli V, van der Flier LG, Cuppen E, van Oudenaarden A, Clevers H, Ascl2 acts as an R-spondin/Wnt-responsive switch to control stemness in intestinal crypts. Cell Stem Cell. 2015 Feb 5;16(2):158-70

- Witkowski MT, Cimmino L, Hu Y, Trimarchi T, Tagoh H, McKenzie MD, Best SA, Tuohey L, Willson TA, Nutt SL, Busslinger M, Aifantis I, Smyth GK, Dickins RA, Activated Notch counteracts Ikaros tumor suppression in mouse and human T-cell acute lymphoblastic leukemia. Leukemia. 2015 Jun;29(6):1301-11

- Yang Z, Balic A, Michon F, Juuri E, Thesleff I, Mesenchymal Wnt/beta-Catenin Signaling Controls Epithelial Stem Cell Homeostasis in Teeth by Inhibiting the Antiapoptotic Effect of Fgf10. Stem Cells. 2015 May;33(5):1670-81

- Liu CY, Dube PE, Girish N, Reddy AT, Polk DB, Optical reconstruction of murine colorectal mucosa at cellular resolution. Am J Physiol Gastrointest Liver Physiol. 2015 May 1;308(9):G721-35

- Carulli AJ, Keeley TM, Demitrack ES, Chung J, Maillard I, Samuelson LC, Notch receptor regulation of intestinal stem cell homeostasis and crypt regeneration. Dev Biol. 2015 Jun 1;402(1):98-108

- Wang T, Chai R, Kim GS, Pham N, Jansson L, Nguyen DH, Kuo B, May LA, Zuo J, Cunningham LL, Cheng AG, Lgr5+ cells regenerate hair cells via proliferation and direct transdifferentiation in damaged neonatal mouse utricle. Nat Commun. 2015;6:6613

- Asfaha S, Hayakawa Y, Muley A, Stokes S, Graham TA, Ericksen RE, Westphalen CB, von Burstin J, Mastracci TL, Worthley DL, Guha C, Quante M, Rustgi AK, Wang TC, Krt19(+)/Lgr5(-) Cells Are Radioresistant Cancer-Initiating Stem Cells in the Colon and Intestine. Cell Stem Cell. 2015 Jun 4;16(6):627- 38

- Chen M, Tian S, Glasgow NG, Gibson G, Yang X, Sh ber CE, Funderburgh J, Watkins S, Johnson JW, Schuman JS, Liu H, Lgr5(+) amacrine cells possess regenerative potential in the retina of adult mice. Aging Cell. 2015 Aug;14(4):635-43

- Fan YY, Davidson LA, Callaway ES, Wright GA, Safe S, Chapkin RS, A bioassay to measure energy metabolism in mouse colonic crypts, organoids, and sorted stem cells. Am J Physiol Gastrointest Liver Physiol. 2015 Jul 1;309(1):G1-9

- Zhao B, Qi Z, Li Y, Wang C, Fu W, Chen YG, The non-muscle-myosin-II heavy chain Myh9 mediates colitis-induced epithelium injury by restricting Lgr5+ stem cells. Nat Commun. 2015;6:7166

- Kuo BR, Baldwin EM, Layman WS, Taketo MM, Zuo J, In Vivo Cochlear Hair Cell Generation and Survival by Coactivation of beta-Catenin and Atoh1. J Neurosci. 2015 Jul 29;35(30):10786-98

- Koren S, Reavie L, Couto JP, De Silva D, Stadler MB, Roloff T, Britschgi A, Eichlisberger T, Kohler H, Aina O, Cardiff RD, Bentires-Alj M, PIK3CA(H1047R) induces multipotency and multi-lineage mammary tumours. Nature. 2015 Sep 3;525(7567):114-8

- Choi E, Petersen CP, Lapierre LA, Williams JA, Weis VG, Goldenring JR, Nam KT, Dynamic expansion of gastric mucosal doublecortin-like kinase 1-expressing cells in response to parietal cell loss is regulated by gastrin. Am J Pathol. 2015 Aug;185(8):2219-31

- Cai J, Maitra A, Anders RA, Taketo MM, Pan D, beta-Catenin destruction complex-independent regulation of Hippo-YAP signaling by APC in intestinal tumorigenesis. Genes Dev. 2015 Jul 15;29(14):1493-506

- Mesa KR, Rompolas P, Zito G, Myung P, Sun TY, Brown S, Gonzalez DG, Blagoev KB, Haberman AM, Greco V, Niche-induced cell death and epithelial phagocytosis regulate hair follicle stem cell pool. Nature. 2015 Jun 4;522(7554):94-7

- DJ, Ridgway RA, Radulescu S, Leushacke M, Campbell AD, Biswas S, Leedham S, Serra S, Chetty R, Moreaux G, Parry L, Matthews J, Song F, Hedley A, Kalna G, Ceteci F, Reed KR, Meniel VS, Maguire A, Doyle B, Soderberg O, Barker N, Watson A, Larue L, Clarke AR, Sansom OJ, E-cadherin can limit the transforming properties of activating beta-catenin mutations. EMBO J. 2015 Sep 14;34(18):2321-33

- Demitrack ES, Gifford GB, Keeley TM, Carulli AJ, VanDussen KL, Thomas D, Giordano TJ, Liu Z, Kopan R, Samuelson LC, Notch signaling regulates gastric antral LGR5 stem cell function. EMBO J. 2015 Oct 14;34(20):2522-36

- DeClercq V, McMurray DN, Chapkin RS, Obesity promotes colonic stem cell expansion during cancer initiation. Cancer Lett. 2015 Dec 28;369(2):336-43

- Morgner J, Ghatak S, Jakobi T, Dieterich C, Aumailley M, Wickstrom SA, Integrin-linked kinase regulates the niche of quiescent epidermal stem cells. Nat Commun. 2015;6:8198

- Davidson LA, Callaway ES, Kim E, Weeks BR, Fan YY, Allred CD, Chapkin RS, Targeted Deletion of p53 in Lgr5-Expressing Intestinal Stem Cells Promotes Colon Tumorigenesis in a Preclinical Model of Colitis-Associated Cancer. Cancer Res. 2015 Dec 15;75(24):5392-7

- Ladang A, Rapino F, Heukamp LC, Tharun L, Shostak K, Hermand D, Delaunay S, Klevernic I, Jiang Z, Jacques N, Jamart D, Migeot V, Florin A, Goktuna S, Malgrange B, Sansom OJ, Nguyen L, Buttner R, Close P, Chariot A, Elp3 drives Wnt-dependent tumor initiation and regeneration in the intestine. J Exp Med. 2015 Nov 16;212(12):2057-75

- Gregorieff A, Liu Y, Inanlou MR, Khomchuk Y, Wrana JL, Yap-dependent reprogramming of Lgr5(+) stem cells drives intestinal regeneration and cancer. Nature. 2015 Oct 29;526(7575):715-8

- Ren W, Lewandowski BC, Watson J, Aihara E, Iwatsuki K, Bachmanov AA, Margolskee RF, Jiang P, Single Lgr5- or Lgr6-expressing taste stem/progenitor cells generate taste bud cells ex vivo. Proc Natl Acad Sci U S A. 2014 Nov 18;111(46):16401-6

- Sheaffer KL, Kim R, Aoki R, Elliott EN, Schug J, Burger L, Schubeler D, Kaestner KH, DNA methylation is required for the control of stem cell differentiation in the small intestine. Genes Dev. 2014 Mar 15;28(6):652-64

- Metcalfe C, K javin NM, Ybarra R, de Sauvage FJ, Lgr5+ stem cells are indispensable for radiation-induced intestinal regeneration. Cell Stem Cell. 2014 Feb 6;14(2):149-59

- Sukhdeo K, Koch CE, Miller TE, Zhou H, Rivera M, Yan K, Cepko CL, Lathia JD, Rich JN, The Lgr5 transgene is expressed specifically in glycinergic amacrine cells in the mouse retina. Exp Eye Res. 2014 Feb;119:106-10

- Hirsch D, Barker N, McNeil N, Hu Y, Camps J, McKinnon K, Clevers H, Ried T, Gaiser T, LGR5 positivity defines stem-like cells in colorectal cancer. Carcinogenesis. 2014 Apr;35(4):849-58

- White AC, Khuu JK, Dang CY, Hu J, Tran KV, Liu A, Gomez S, Zhang Z, Yi R, Scumpia P, Grigorian M, Lowry WE, Stem cell quiescence acts as a tumour suppressor in squamous tumours. Nat Cell Biol. 2014 Jan;16(1):99-107

- Cox BC, Chai R, Lenoir A, Liu Z, Zhang L, Nguyen DH, Chalasani K, Steigelman KA, Fang J, Rubel EW, Cheng AG, Zuo J, Spontaneous hair cell regeneration in the neonatal mouse cochlea in vivo. Development. 2014 Feb;141(4):816-29

- Ritsma L, Ellenbroek SI, Zomer A, Snippert HJ, de Sauvage FJ, Simons BD, Clevers H, van Rheenen J, Intestinal crypt homeostasis revealed at single-stem-cell level by in vivo live imaging. Nature. 2014 Mar 20;507(7492):362-5

- Perekatt AO, Valdez MJ, Davila M, Hoffman A, Bonder EM, Gao N, Verzi MP, YY1 is indispensable for Lgr5+ intestinal stem cell renewal. Proc Natl Acad Sci U S A. 2014 May 27;111(21):7695-700

- Wiener Z, Band AM, Kallio P, Hogstrom J, Hyvonen V, Kaijalainen S, Ritvos O, Haglund C, Kruuna O, Robine S, Louvard D, Ben-Neriah Y, Alitalo K, Oncogenic mutations in intestinal adenomas regulate Bim-mediated apoptosis induced by TGF-beta. Proc Natl Acad Sci U S A. 2014 May 27;111(21):E2229-36

- Nakaya T, Ogawa S, Manabe I, Tanaka M, Sanada M, Sato T, Taketo MM, Nakao K, Clevers H, Fukayama M, Kuroda M, Nagai R, KLF5 regulates the integrity and oncogenicity of intestinal stem cells. Cancer Res. 2014 May 15;74(10):2882-91

- Cordero JB, Ridgway RA, Valeri N, Nixon C, Frame MC, Muller WJ, Vidal M, Sansom OJ, c-Src drives intestinal regeneration and transformation. EMBO J. 2014 Jul 1;33(13):1474-91

- Chen M, Tian S, Yang X, Lane AP, Reed RR, Liu H, Wnt-responsive Lgr5(+) globose basal cells function as multipotent olfactory epithelium progenitor cells. J Neurosci. 2014 Jun 11;34(24):8268-76

- Boumahdi S, Driessens G, Lapouge G, Rorive S, Nassar D, Le Mercier M, Delatte B, Caauwe A, Lenglez S, Nkusi E, Brohee S, Salmon I, Dubois C, del Marmol V, Fuks F, Beck B, Blanpain C, SOX2 controls tumour initiation and cancer stem-cell functions in squamous-cell carcinoma. Nature. 2014 Jul 10;511(7508):246-50

GPR50

Knockout references

http://www.mousephenotype.org/data/genes/MGI:1333877
http://www.informatics.jax.org/marker/MGI:1333877

Phenotype references

- Wattanachanya L, Lu WD, Kundu RK, Wang L, Abbott MJ, O'Carroll D, Quertermous T, Nissenson RA, Increased bone mass in mice lacking the adipokine apelin. Endocrinology. 2013 Jun;154(6):2069-80

- Bechtold DA, Sidibe A, Saer BR, Li J, Hand LE, Ivanova EA, Darras VM, Dam J, Jockers R, Luckman SM, Loudon AS, A Role for the Melatonin-Related Receptor GPR50 in Leptin Signaling, Adaptive Thermogenesis, and Torpor. Curr Biol. 2012 Jan10;22(1):70-7

- Li J, Hand LE, Meng QJ, Loudon AS, Bechtold DA, GPR50 interacts with TIP60 to modulate glucocorticoid receptor signalling. PLoS One. 2011;6(8):e23725

- Skarnes WC, Rosen B, West AP, Koutsourakis M, Bushell W, Iyer V, Mujica AO, Thomas M, Harrow J, Cox T, Jackson D, Severin J, Biggs P, Fu J, Nefedov M, de Jong PJ, Stewart AF, Bradley A, A conditional knockout resource for the genome-wide study of mouse gene function. Nature. 2011 Jun 16;474(7351):337- 42

- Ivanova EA, Bechtold DA, Dupre SM, Brennand J, Barrett P, Luckman SM, Loudon AS, Altered metabolism in the melatonin-related receptor (GPR50) knockout mouse. Am J Physiol Endocrinol Metab. 2008 Jan;294(1):E176-82

- Barrett P, Ivanova E, Graham ES, Ross AW, Wilson D, Ple H, Mercer JG, Ebling FJ, Schuhler S, Dupre SM, Loudon A, Morgan PJ, Photoperiodic regulation of cellular retinoic acid-binding protein 1, GPR50 and nestin in tanycytes of the third ventricle ependymal layer of the Siberian hamster. J Endocrinol. 2006 Dec;191(3):687-98

GPR51

Knockout references

http://www.mousephenotype.org/data/genes/MGI:2386030
http://www.informatics.jax.org/marker/MGI:2386030

Phenotype references

- Hanack C, Moroni M, Lima WC, Wende H, Kirchner M, Adelfinger L, Schrenk-Siemens K, Tappe-Theodor A, Wetzel C, Kuich PH, Gassmann M, Roggenkamp D, Bettler B, Lewin GR, Selbach M, Siemens J, GABA blocks pathological but not acute TRPV1 pain signals. Cell. 2015 Feb 12;160(4):759-70

- Turecek R, Schwenk J, Fritzius T, Ivankova K, Zolles G, Adelfinger L, Jacquier V, Besseyrias V, Gassmann M, Schulte U, Fakler B, Bettler B, Auxiliary GABAB receptor subunits uncouple G protein betagamma subunits from effector channels to induce desensitization. Neuron. 2014 Jun 4;82(5):1032-44

- Giachino C, Barz M, Tchorz JS, Tome M, Gassmann M, Bischofberger J, Bettler B, Taylor V, GABA suppresses neurogenesis in the adult hippocampus through GABAB receptors. Development. 2014 Jan;141(1):83-90

- Terunuma M, Revilla-Sanchez R, Quadros IM, Deng Q, Deeb TZ, Lumb M, Sicinski P, Haydon PG, Pangalos MN, Moss SJ, Postsynaptic GABAB Receptor Activity Regulates Excitatory Neuronal Architecture and Spatial Memory. J Neurosci. 2014 Jan 15;34(3):804-16

- Adelfinger L, Turecek R, Ivankova K, Jensen AA, Moss SJ, Gassmann M, Bettler B, GABAB receptor phosphorylation regulates KCTD12-induced K(+) current desensitization. Biochem Pharmacol. 2014 Oct 1;91(3):369-79

- Vienne J, Bettler B, Franken P, Tafti M, Differential effects of GABAB receptor subtypes, {gamma}-hydroxybutyric Acid, and Baclofen on EEG activity and sleep regulation. J Neurosci. 2010 Oct 20;30(42):14194-204

- Hansen GM, Markesich DC, Burnett MB, Zhu Q, Dionne KM, Richter LJ, Finnell RH, Sands AT, Zambrowicz BP, Abuin A, Large-scale gene trapping in C57BL/6N mouse embryonic stem cells. Genome Res. 2008 Oct;18(10):1670-9

- Mombereau C, Kaupmann K, Gassmann M, Bettler B, van der Putten H, Cryan JF, Altered anxiety and depression-related behaviour in mice lacking GABAB(2) receptor subunits. Neuroreport. 2005 Feb 28;16(3):307-10

- Thuault SJ, Brown JT, Sheardown SA, Jourdain S, Fairfax B, Spencer JP, Restituito S, Nation JH, Topps S, Medhurst AD, Randall AD, Couve A, Moss SJ, Collingridge GL, Pangalos MN, Davies CH, Calver AR, The GABA(B2) subunit is critical for the trafficking and function of native GABA(B) receptors. Biochem Pharmacol. 2004 Oct 15;68(8):1655-66

- Gassmann M, Shaban H, Vigot R, Sansig G, Haller C, Barbieri S, Humeau Y, Schuler V, Muller M, Kinzel B, Klebs K, Schmutz M, Froestl W, Heid J, Kelly PH, Gentry C, Jaton AL, Van der Putten H, Mombereau C, Lecourtier L, Mosbacher J, Cryan JF, Fritschy JM, Luthi A, Kaupmann K, Bettler B, Redistr bution of GABAB(1) protein and atypical GABAB responses in GABAB(2)-deficient mice. J Neurosci. 2004 Jul 7;24(27):6086-97

- Zambrowicz BP, Abuin A, Ramirez-Solis R, Richter LJ, Piggott J, BeltrandelRio H, Buxton EC, Edwards J, Finch RA, Friddle CJ, Gupta A, Hansen G, Hu Y, Huang W, Jaing C, Key BW Jr, Kipp P, Kohlhauff B, Ma ZQ, Markesich D, Payne R, Potter DG, Qian N, Shaw J, Schrick J, Shi ZZ, Sparks MJ, Van Sligtenhorst I, Vogel P, Walke W, Xu N, Zhu Q, Person C, Sands AT, Wnk1 kinase deficiency lowers blood pressure in mice: a gene-trap screen to identify potential targets for therapeutic intervention. Proc Natl Acad Sci U S A. 2003 Nov 25;100(24):14109-14

GPR54

Knockout references

http://www.informatics.jax.org/marker/MGI:2148793

Phenotype references

- Herreboudt AM, Kyle VR, Lawrence J, Doran J, Colledge WH, Kiss1 mutant placentas show normal structure and function in the mouse. Placenta. 2015 Jan;36(1):52-8

- Navarro VM, Bosch MA, Leon S, Simavli S, True C, Pinilla L, Carroll RS, Seminara SB, Tena-Sempere M, Ronnekleiv OK, Kaiser UB, The integrated hypothalamic tachykinin-kisspeptin system as a central coordinator for reproduction. Endocrinology. 2015 Feb;156(2):627-37

- Liu X, Herbison A, Kisspeptin regulation of arcuate neuron excitability in kisspeptin receptor knockout mice. Endocrinology. 2015 May;156(5):1815-27

- Kumar D, Periasamy V, Freese M, Voigt A, Boehm U, In Utero Development of Kisspeptin/GnRH Neural Circuitry in Male Mice. Endocrinology. 2015 Sep;156(9):3084-90

- Song WJ, Mondal P, Wolfe A, Alonso LC, Stamateris R, Ong BW, Lim OC, Yang KS, Radovick S, Novaira HJ, Farber EA, Farber CR, Turner SD, Hussain MA, Glucagon regulates hepatic kisspeptin to impair insulin secretion. Cell Metab. 2014 Apr 1;19(4):667-81

- Novaira HJ, Sonko ML, Hoffman G, Koo Y, Ko C, Wolfe A, Radovick S, Disrupted kisspeptin signaling in GnRH neurons leads to hypogonadotrophic hypogonadism. Mol Endocrinol. 2014 Feb;28(2):225-38

- Bellefontaine N, Chachlaki K, Parkash J, Vanacker C, Colledge W, d'Anglemont de Tassigny X, Garthwaite J, Bouret SG, Prevot V, Leptin-dependent neuronal NO signaling in the preoptic hypothalamus facilitates reproduction. J Clin Invest. 2014 Jun 2;124(6):2550-9

- Gaytan F, Garcia-Galiano D, Dorfman MD, Manfredi-Lozano M, Castellano JM, Dissen GA, Ojeda SR, Tena-Sempere M, Kisspeptin receptor haplo-insufficiency causes premature ovarian failure despite preserved gonadotropin secretion. Endocrinology. 2014 Aug;155(8):3088-97

- Dorfman MD, Garcia-Rudaz C, Alderman Z, Kerr B, Lomniczi A, Dissen GA, Castellano JM, Garcia-Galiano D, Gaytan F, Xu B, Tena-Sempere M, Ojeda SR, Loss of Ntrk2/Kiss1r signaling in oocytes causes premature ovarian failure. Endocrinology. 2014 Aug;155(8):3098-111

- Calder M, Chan YM, Raj R, Pampillo M, Elbert A, Noonan M, Gillio-Meina C, Caligioni C, Berube NG, Bhattacharya M, Watson AJ, Seminara SB, Babwah AV, Implantation failure in female Kiss1-/- mice is independent of their hypogonadic state and can be partially rescued by leukemia inhibitory factor. Endocrinology. 2014 Aug;155(8):3065-78

- Kumar D, Freese M, Drexler D, Hermans-Borgmeyer I, Marquardt A, Boehm U, Murine arcuate nucleus kisspeptin neurons communicate with GnRH neurons in utero. J Neurosci. 2014 Mar 5;34(10):3756-66

- Tolson KP, Garcia C, Yen S, Simonds S, Stefanidis A, Lawrence A, Smith JT, Kauffman AS, Impaired kisspeptin signaling decreases metabolism and promotes glucose intolerance and obesity. J Clin Invest. 2014 Jul 1;124(7):3075-9

- Gaytan F, Sangiao-Alvarellos S, Manfredi-Lozano M, Garcia-Galiano D, Ruiz-Pino F, Romero-Ruiz A, Leon S, Morales C, Cordido F, Pinilla L, Tena-Sempere M, Distinct expression patterns predict differential roles of the miRNA-binding proteins, Lin28 and Lin28b, in the mouse testis: studies during postnatal development and in a model of hypogonadotropic hypogonadism. Endocrinology. 2013 Mar;154(3):1321-36

- Kirilov M, Clarkson J, Liu X, Roa J, Campos P, Porteous R, Schutz G, Herbison AE, Dependence of fertility on kisspeptin-Gpr54 signaling at the GnRH neuron. Nat Commun. 2013;4:2492

- Dror T, Franks J, Kauffman AS, Analysis of multiple positive feedback paradigms demonstrates a complete absence of LH surges and GnRH activation in mice lacking kisspeptin signaling. Biol Reprod. 2013 Jun;88(6):146

- Steyn FJ, Wan Y, Clarkson J, Veldhuis JD, Herbison AE, Chen C, Development of a methodology for and assessment of pulsatile luteinizing hormone secretion in juvenile and adult male mice. Endocrinology. 2013 Dec;154(12):4939-45

- Hanchate NK, Parkash J, Bellefontaine N, Mazur D, Colledge WH, d'Anglemont de Tassigny X, Prevot V, Kisspeptin-GPR54 Signaling in Mouse NO-Synthesizing Neurons Participates in the Hypothalamic Control of Ovulation. J Neurosci. 2012 Jan 18;32(3):932-45

- Witham EA, Meadows JD, Shojaei S, Kauffman AS, Mellon PL, Prenatal exposure to low levels of androgen accelerates female puberty onset and reproductive senescence in mice. Endocrinology. 2012 Sep;153(9):4522-32

- Garcia-Galiano D, van Ingen Schenau D, Leon S, Krajnc-Franken MA, Manfredi-Lozano M, Romero-Ruiz A, Navarro VM, Gaytan F, van Noort PI, Pinilla L, Blomenrohr M, Tena-Sempere M, Kisspeptin signaling is indispensable for neurokinin B, but not glutamate, stimulation of gonadotropin secretion in mice. Endocrinology. 2012 Jan;153(1):316-28

- Poling MC, Kauffman AS, Sexually dimorphic testosterone secretion in prenatal and neonatal mice is independent of kisspeptin-Kiss1r and GnRH signaling. Endocrinology. 2012 Feb;153(2):782-93

- Cho SG, Wang Y, Rodriguez M, Tan K, Zhang W, Luo J, Li D, Liu M, Haploinsufficiency in the prometastasis kiss1 receptor gpr54 delays breast tumor initiation, progression, and lung metastasis. Cancer Res. 2011 Oct 15;71(20):6535-46

- Liu X, Porteous R, d'Anglemont de Tassigny X, Colledge WH, Millar R, Petersen SL, Herbison AE, Frequency-Dependent Recruitment of Fast Amino Acid and Slow Neuropeptide Neurotransmitter Release Controls Gonadotropin-Releasing Hormone Neuron Excitability. J Neurosci. 2011 Feb 16;31(7):2421-2430

- Prentice LM, d'Anglemont de Tassigny X, McKinney S, Ruiz de Algara T, Yap D, Turashvili G, Poon S, Sutcliffe M, Allard P, Burleigh A, Fee J, Huntsman DG, Colledge WH, Aparicio SA, The testosterone- dependent and independent transcriptional networks in the hypothalamus of Gpr54 and Kiss1 knockout male mice are not fully equivalent. BMC Genomics. 2011;12:209

- Mayer C, Boehm U, Female reproductive maturation in the absence of kisspeptin/GPR54 signaling. Nat Neurosci. 2011 Jun;14(6):704-10

- Mei H, Walters C, Carter R, Colledge WH, Gpr54-/- mice show more pronounced defects in spermatogenesis than Kiss1-/- mice and improved spermatogenesis with age when exposed to dietary phytoestrogens. Reproduction. 2011 Mar;141(3):357-66

- Maguire JJ, Kirby HR, Mead EJ, Kuc RE, d'Anglemont de Tassigny X, Colledge WH, Davenport AP, Inotropic action of the puberty hormone kisspeptin in rat, mouse and human: cardiovascular distribution and characteristics of the kisspeptin receptor. PLoS One. 2011;6(11):e27601

- Tuomi JM, Chidiac P, Jones DL, Evidence for enhanced M3 muscarinic receptor function and sensitivity to atrial arrhythmia in the RGS2-deficient mouse. Am J Physiol Heart Circ Physiol. 2010 Feb;298(2):H554-61

- d'Anglemont de Tassigny X, Ackroyd KJ, Chatzidaki EE, Colledge WH, Kisspeptin signaling is required for peripheral but not central stimulation of gonadotropin-releasing hormone neurons by NMDA. J Neurosci. 2010 Jun 23;30(25):8581-90

- Yi T, Tan K, Cho SG, Wang Y, Luo J, Zhang W, Li D, Liu M, Regulation of embryonic kidney branching morphogenesis and glomerular development by KISS1 receptor (Gpr54) through NFAT2- and Sp1-mediated Bmp7 expression. J Biol Chem. 2010 Jun 4;285(23):17811-20

- d'Anglemont de Tassigny X, Fagg LA, Carlton MB, Colledge WH, Kisspeptin can stimulate gonadotropin-releasing hormone (GnRH) release by a direct action at GnRH nerve terminals. Endocrinology. 2008 Aug;149(8):3926-32

- Clarkson J, d'Anglemont de Tassigny X, Moreno AS, Colledge WH, Herbison AE, Kisspeptin-GPR54 signaling is essential for preovulatory gonadotropin-releasing hormone neuron activation and the luteinizing hormone surge. J Neurosci. 2008 Aug 27;28(35):8691-7

- Kauffman AS, Park JH, McPhie-Lalmansingh AA, Gottsch ML, Bodo C, Hohmann JG, Pavlova MN, Rohde AD, Clifton DK, Steiner RA, Rissman EF, The kisspeptin receptor GPR54 is required for sexual differentiation of the brain and behavior. J Neurosci. 2007 Aug 15;27(33):8826-35

- Dungan HM, Gottsch ML, Zeng H, Gragerov A, Bergmann JE, Vassilatis DK, Clifton DK, Steiner RA, The role of kisspeptin-GPR54 signaling in the tonic regulation and surge release of gonadotropin-releasing hormone/luteinizing hormone. J Neurosci. 2007 Oct 31;27(44):12088-95

- Lapatto R, Pallais JC, Zhang D, Chan YM, Mahan A, Cerrato F, Le WW, Hoffman GE, Seminara SB, Kiss1-/- mice exhibit more variable hypogonadism than Gpr54-/- mice. Endocrinology. 2007 Oct;148(10):4927-36

- Messager S, Chatzidaki EE, Ma D, Hendrick AG, Zahn D, Dixon J, Thresher RR, Malinge I, Lomet D, Carlton MB, Colledge WH, Caraty A, Aparicio SA, Kisspeptin directly stimulates gonadotropin-releasing hormone release via G protein-coupled receptor 54. Proc Natl Acad Sci U S A. 2005 Feb 1;102(5):1761-6

- Seminara SB, Messager S, Chatzidaki EE, Thresher RR, Acierno JS Jr, Shagoury JK, Bo-Abbas Y, Kuohung W, Schwinof KM, Hendrick AG, Zahn D, Dixon J, Kaiser UB, Slaugenhaupt SA, Gusella JF, O'Rahilly S, Carlton MB, Crowley WF Jr, Aparicio SA, Colledge WH, The GPR54 gene as a regulator of puberty. N Engl J Med. 2003 Oct 23;349(17):1614-27

- Funes S, Hedrick JA, Vassileva G, Markowitz L, Abbondanzo S, Golovko A, Yang S, Monsma FJ, Gustafson EL, The KiSS-1 receptor GPR54 is essential for the development of the murine reproductive system. Biochem Biophys Res Commun. 2003 Dec 26;312(4):1357-63

- Benson KF, Chada K, Molecular Characterization of the Mouse In(10)17Rk Inversion and Identification of a Novel Muscle-Specific Gene at the Proximal Breakpoint. Genetics. 2002 Jan;160(1):279-87

- Lyon MF, Rastan S, Brown SDM, in Lyon MF, Rastan S, Brown SDM (eds), Genetic Variants and Strains of the Laboratory Mouse. 3rd Edition. 1996Oxford: Oxford University Press.

- Zhou X, Benson KF, Ashar HR, Chada K, Mutation respons ble for the mouse pygmy phenotype in the developmentally regulated factor HMGI-C [see comments]. Nature. 1995 Aug 31;376(6543):771-4

- Roderick TH, Using inversions to detect and study recessive lethals and detrimentals in mice, in de Serres FJ, Sheridan W (eds), Utilization of Mammalian Specific Locus Studies in Hazard Evaluation and Estimation of Genetic Risk. 1983:135-67. Plenum Publishing Corp..

- Roderick TH, Chromosomal inversions in studies of mammalian mutagenesis. Genetics. 1979 May;92(1 Pt 1 Suppl):s121-6

GPR55

Knockout references

http://www.mousephenotype.org/data/genes/MGI:2685064;
http://www.informatics.jax.org/allele/MGI:3528724?page=alleleDetail&id=MGI:3528724

Phenotype references

- Wu CS, Chen H, Sun H, Zhu J, Jew CP, Wager-Miller J, Straiker A, Spencer C, Bradshaw H, Mackie K, Lu HC, GPR55, a G-protein coupled receptor for lysophosphatidylinositol, plays a role in motor coordination. PLoS One. 2013;8(4):e60314

- Sisay S, Pryce G, Jackson SJ, Tanner C, Ross RA, Michael GJ, Selwood DL, Giovannoni G, Baker D, Genetic background can result in a marked or minimal effect of gene knockout (GPR55 and CB2 receptor) in experimental autoimmune encephalomyelitis models of multiple sclerosis. PLoS One. 2013;8(10):e76907

- Li K, Fichna J, Schicho R, Saur D, Bashashati M, Mackie K, Li Y, Zimmer A, Goke B, Sharkey KA, Storr M, A role for O-1602 and G protein-coupled receptor GPR55 in the control of colonic motility in mice. Neuropharmacology. 2013 Aug;71:255-63

- Schicho R, Bashashati M, Bawa M, McHugh D, Saur D, Hu HM, Zimmer A, Lutz B, Mackie K, Bradshaw HB, McCafferty DM, Sharkey KA, Storr M, The atypical cannabinoid O-1602 protects against experimental colitis and inhibits neutrophil recruitment. Inflamm Bowel Dis. 2011 Aug;17(8):1651-64

GPR56

Knockout references

http://www.mousephenotype.org/data/genes/MGI:1340051

http://www.informatics.jax.org/marker/MGI:1340051

Phenotype references

- White JP, Wrann CD, Rao RR, Nair SK, Jedrychowski MP, You JS, Martinez-Redondo V, Gygi SP, Ruas JL, Hornberger TA, Wu Z, Glass DJ, Piao X, Spiegelman BM, G protein-coupled receptor 56 regulates mechanical overload-induced muscle hypertrophy. Proc Natl Acad Sci U S A. 2014 Nov 4;111(44):15756-61

- Saito Y, Kaneda K, Suekane A, Ichihara E, Nakahata S, Yamakawa N, Nagai K, Mizuno N, Kogawa K, Miura I, Itoh H, Morishita K, Maintenance of the hematopoietic stem cell pool in bone marrow niches by EVI1-regulated GPR56. Leukemia. 2013 Aug;27(8):1637-49

- Wu MP, Doyle JR, Barry B, Beauvais A, Rozkalne A, Piao X, Lawlor MW, Kopin AS, Walsh CA, Gussoni E, G-protein coupled receptor 56 promotes myoblast fusion through serum response factor- and nuclear factor of activated T-cell-mediated signalling but is not essential for muscle development in vivo. FEBS J. 2013 Dec;280(23):6097-113

- Jeong SJ, Luo R, Singer K, Giera S, Kreidberg J, Kiyozumi D, Shimono C, Sekiguchi K, Piao X, GPR56 functions together with alpha3beta1 integrin in regulating cerebral cortical development. PLoS One. 2013;8(7):e68781

- Jeong SJ, Luo R, Li S, Strokes N, Piao X, Characterization of G protein-coupled receptor 56 protein expression in the mouse developing neocortex. J Comp Neurol. 2012 Sep 1;520(13):2930-40

- Yang L, Chen G, Mohanty S, Scott G, Fazal F, Rahman A, Begum S, Hynes RO, Xu L, GPR56 Regulates VEGF Production and Angiogenesis during Melanoma Progression. Cancer Res. 2011 Aug 15;71(16):5558-68

- Chen G, Yang L, Begum S, Xu L, GPR56 is essential for testis development and male fertility in mice. Dev Dyn. 2010 Dec;239(12):3358-67

- Koirala S, Jin Z, Piao X, Corfas G, GPR56-regulated granule cell adhesion is essential for rostral cerebellar development. J Neurosci. 2009 Jun 10;29(23):7439-49

- Li S, Jin Z, Koirala S, Bu L, Xu L, Hynes RO, Walsh CA, Corfas G, Piao X, GPR56 regulates pial basement membrane integrity and cortical lamination. J Neurosci. 2008 May 28;28(22):5817-26

- Zambrowicz BP, Abuin A, Ramirez-Solis R, Richter LJ, Piggott J, BeltrandelRio H, Buxton EC, Edwards J, Finch RA, Friddle CJ, Gupta A, Hansen G, Hu Y, Huang W, Jaing C, Key BW Jr, Kipp P, Kohlhauff B, Ma ZQ, Markesich D, Payne R, Potter DG, Qian N, Shaw J, Schrick J, Shi ZZ, Sparks MJ, Van Sligtenhorst I, Vogel P, Walke W, Xu N, Zhu Q, Person C, Sands AT, Wnk1 kinase deficiency lowers blood pressure in mice: a gene-trap screen to identify potential targets for therapeutic intervention. Proc Natl Acad Sci U S A. 2003 Nov 25;100(24):1410 9-14

GPR64

Knockout references

http://www.mousephenotype.org/data/genes/MGI:2446854
http://www.informatics.jax.org/marker/MGI:2446854

Phenotype references

- Skarnes WC, Rosen B, West AP, Koutsourakis M, Bushell W, Iyer V, Mujica AO, Thomas M, Harrow J, Cox T, Jackson D, Severin J, Biggs P, Fu J, Nefedov M, de Jong PJ, Stewart AF, Bradley A, A conditional knockout resource for the genome-wide study of mouse gene function. Nature. 2011 Jun 16;474(7351):337- 42

- Davies B, Behnen M, Cappallo-Obermann H, Spiess AN, Theuring F, Kirchhoff C, Novel epididymis-specific mRNAs downregulated by HE6/Gpr64 receptor gene disruption. Mol Reprod Dev. 2007 May;74(5):539-53

- Davies B, Baumann C, Kirchhoff C, Ivell R, Nubbemeyer R, Habenicht UF, Theuring F, Gottwald U, Targeted deletion of the epididymal receptor HE6 results in fluid dysregulation and male infertility. Mol Cell Biol. 2004 Oct;24(19):8642-8

- Blair HJ, Gormally E, Uwechue IC, Boyd Y, Mouse mutants carrying deletions that remove the genes mutated in Coffin-Lowry syndrome and lactic acidosis. Hum Mol Genet. 1998 Mar;7(3):549-55

GPR65

Knockout references

http://www.mousephenotype.org/data/genes/MGI:108031
http://www.informatics.jax.org/marker/MGI:108031

Phenotype references

- Hikiji H, Endo D, Horie K, Harayama T, Akahoshi N, Igarashi H, Kihara Y, Yanagida K, Takeda J, Koji T, Shimizu T, Ishii S, TDAG8 activation inhibits osteoclastic bone resorption. FASEB J. 2014 Feb;28(2):871-9

- Jin Y, Sato K, Tobo A, Mogi C, Tobo M, Murata N, Ishii S, Im DS, Okajima F, Inh bition of interleukin-1beta production by extracellular acidification through the TDAG8/cAMP pathway in mouse microglia. J Neurochem. 2014 May;129(4):683-95

- Zhu X, Mose E, Hogan SP, Zimmermann N, Differential eosinophil and mast cell regulation: mast cell viability and accumulation in inflammatory tissue are independent of proton-sensing receptor GPR65. Am J Physiol Gastrointest Liver Physiol. 2014 Jun 1;306(11):G974-82

- He XD, Tobo M, Mogi C, Nakakura T, Komachi M, Murata N, Takano M, Tomura H, Sato K, Okajima F, Involvement of proton-sensing receptor TDAG8 in the anti-inflammatory actions of dexamethasone in peritoneal macrophages. Biochem Biophys Res Commun. 2011 Dec 2;415(4):627-31

- Skarnes WC, Rosen B, West AP, Koutsourakis M, Bushell W, Iyer V, Mujica AO, Thomas M, Harrow J, Cox T, Jackson D, Severin J, Biggs P, Fu J, Nefedov M, de Jong PJ, Stewart AF, Bradley A, A conditional knockout resource for the genome-wide study of mouse gene function. Nature. 2011 Jun 16;474(7351):337- 42

- Mogi C, Tobo M, Tomura H, Murata N, He XD, Sato K, Kimura T, Ishizuka T, Sasaki T, Sato T, Kihara Y, Ishii S, Harada A, Okajima F, Involvement of proton-sensing TDAG8 in extracellular acidification-induced inhibition of proinflammatory cytokine production in peritoneal macrophages. J Immunol. 2009 Mar 1;182(5):3243-51

- Radu CG, Cheng D, Nijagal A, Riedinger M, McLaughlin J, Yang LV, Johnson J, Witte ON, Normal immune development and glucocorticoid-induced thymocyte apoptosis in mice deficient for the T-cell death-associated gene 8 receptor. Mol Cell Biol. 2006 Jan;26(2):668-77

- Radu CG, Nijagal A, McLaughlin J, Wang L, Witte ON, Differential proton sensitivity of related G protein-coupled receptors T cell death-associated gene 8 and G2A expressed in immune cells. Proc Natl Acad Sci U S A. 2005 Feb 1;102(5):1632-7

- Lyon MF, Rastan S, Brown SDM, in Lyon MF, Rastan S, Brown SDM (eds), Genetic Variants and Strains of the Laboratory Mouse. 3rd Edition. 1996Oxford: Oxford University Press.

- Roderick TH, Using inversions to detect and study recessive lethals and detrimentals in mice, in de Serres FJ, Sheridan W (eds), Utilization of Mammalian Specific Locus Studies in Hazard Evaluation and Estimation of Genetic Risk. 1983:135-67. Plenum Publishing Corp..

GPR66

Knockout references

http://www.mousephenotype.org/data/genes/MGI:1341898
http://www.informatics.jax.org/marker/MGI:1341898

Phenotype references

- Peier AM, Desai K, Hubert J, Du X, Yang L, Qian Y, Kosinski JR, Metzger JM, Pocai A, Nawrocki AR, Langdon RB, Marsh DJ, Effects of peripherally administered neuromedin u on energy and glucose homeostasis. Endocrinology. 2011Jul;152(7):2644-54

- Peier A, Kosinski J, Cox-York K, Qian Y, Desai K, Feng Y, Trivedi P, Hastings N, Marsh DJ, The antiobesity effects of centrally administered neuromedin U and neuromedin S are mediated predominantly by the neuromedin U receptor 2 (NMUR2). Endocrinology. 2009 Jul;150(7):3101-9

- Abbondanzo SJ, Manfra DJ, Chen SC, Pinzon-Ortiz M, Sun Y, Phillips JE, Laverty M, Vassileva G, Hu W, Yang S, Gustafson EL, Fine JS, Hedrick JA, Nmur1-/- mice are not protected from cutaneous inflammation. Biochem Biophys Res Commun. 2009 Jan 23;378(4):777-82

- Torres R, Croll SD, Vercollone J, Reinhardt J, Griffiths J, Zabski S, Anderson KD, Adams NC, Gowen L, Sleeman MW, Valenzuela DM, Wiegand SJ, Yancopoulos GD, Murphy AJ, Mice genetically deficient in neuromedin U receptor 2, but not neuromedin U receptor 1, have impaired nociceptive responses. Pain. 2007 Aug;130(3):267-78

- Prendergast CE, Morton MF, Figueroa KW, Wu X, Shankley NP, Species-dependent smooth muscle contraction to Neuromedin U and determination of the receptor subtypes mediating contraction using NMU1 receptor knockout mice. Br J Pharmacol. 2006 Apr;147(8):886-96

- Valenzuela DM, Murphy AJ, Frendewey D, Gale NW, Economides AN, Auerbach W, Poueymirou WT, Adams NC, Rojas J, Yasenchak J, Chernomorsky R, Boucher M, Elsasser AL, Esau L, Zheng J, Griffiths JA, Wang X, Su H, Xue Y, Dominguez MG, Noguera I, Torres R, Macdonald LE, Stewart AF, DeChiara TM, Yancopoulos GD, High-throughput engineering of the mouse genome coupled with high-resolution expression analysis. Nat Biotechnol. 2003Jun;21(6):652-9

- Lyon MF, Rastan S, Brown SDM, in Lyon MF, Rastan S, Brown SDM (eds), Genetic Variants and Strains of the Laboratory Mouse. 3rd Edition. 1996Oxford: Oxford University Press.

- Juriloff DM, Sulik KK, Roderick TH, Hogan BK, Genetic and developmental studies of a new mouse mutation that produces otocephaly. J Craniofac Genet Dev Biol. 1985;5(2):121-45

- Roderick TH, Using inversions to detect and study recessive lethals and detrimentals in mice, in de Serres FJ, Sheridan W (eds), Utilization of Mammalian Specific Locus Studies in Hazard Evaluation and Estimation of Genetic Risk. 1983:135-67. Plenum Publishing Corp..

- Roderick TH, Hawes NL, Two radiation-induced chromosomal inversions in mice (Mus musculus). Proc Natl Acad Sci U S A. 1970 Oct;67(2):961-7

GPR68

Knockout references

http://www.mousephenotype.org/data/genes/MGI:2441763
http://www.informatics.jax.org/marker/MGI:2441763

Phenotype references

- Aoki H, Mogi C, Hisada T, Nakakura T, Kamide Y, Ichimonji I, Tomura H, Tobo M, Sato K, Tsurumaki H, Dobashi K, Mori T, Harada A, Yamada M, Mori M, Ishizuka T, Okajima F, Proton-sensing ovarian cancer G protein-coupled receptor 1 on dendritic cells is required for airway responses in a murine asthma model. PLoS One. 2013;8(11):e79985

- Nakakura T, Mogi C, Tobo M, Tomura H, Sato K, Kobayashi M, Ohnishi H, Tanaka S, Wayama M, Sugiyama T, Kitamura T, Harada A, Okajima F, Deficiency of proton-sensing ovarian cancer G protein-coupled receptor 1 attenuates glucose-stimulated insulin secretion. Endocrinology. 2012 Sep;153(9):4171-80

- Li H, Wang D, Singh LS, Berk M, Tan H, Zhao Z, Steinmetz R, Kirmani K, Wei G, Xu Y, Abnormalities in osteoclastogenesis and decreased tumorigenesis in mice deficient for ovarian cancer G protein-coupled receptor 1. PLoS One. 2009;4(5):e5705

- Mogi C, Tobo M, Tomura H, Murata N, He XD, Sato K, Kimura T, Ishizuka T, Sasaki T, Sato T, Kihara Y, Ishii S, Harada A, Okajima F, Involvement of proton-sensing TDAG8 in extracellular acidification-induced inhibition of proinflammatory cytokine production in peritoneal macrophages. J Immunol. 2009 Mar 1;182(5):3243-51

- Lyon MF, Rastan S, Brown SDM, in Lyon MF, Rastan S, Brown SDM (eds), Genetic Variants and Strains of the Laboratory Mouse. 3rd Edition. 1996Oxford: Oxford University Press.

- Roderick TH, Using inversions to detect and study recessive lethals and detrimentals in mice, in de Serres FJ, Sheridan W (eds), Utilization of Mammalian Specific Locus Studies in Hazard Evaluation and Estimation of Genetic Risk. 1983:135-67. Plenum Publishing Corp..

GPR69

Knockout references

http://www.mousephenotype.org/data/genes/MGI:1336997
http://www.informatics.jax.org/marker/MGI:1336997

Phenotype references

- Huang C, Chen M, Pang D, Bi D, Zou Y, Xia X, Yang W, Luo L, Deng R, Tan H, Zhou L, Yu S, Guo L, Du X, Cui Y, Hu J, Mao Q, Worley PF, Xiao B, Developmental and activity-dependent expression of LanCL1 confers antioxidant activity required for neuronal survival. Dev Cell. 2014 Aug 25;30(4):479-87

- Skarnes WC, Rosen B, West AP, Koutsourakis M, Bushell W, Iyer V, Mujica AO, Thomas M, Harrow J, Cox T, Jackson D, Severin J, Biggs P, Fu J, Nefedov M, de Jong PJ, Stewart AF, Bradley A, A conditional knockout resource for the genome-wide study of mouse gene function. Nature. 2011 Jun 16;474(7351):337- 42

- Zambrowicz BP, Abuin A, Ramirez-Solis R, Richter LJ, Piggott J, BeltrandelRio H, Buxton EC, Edwards J, Finch RA, Friddle CJ, Gupta A, Hansen G, Hu Y, Huang W, Jaing C, Key BW Jr, Kipp P, Kohlhauff B, Ma ZQ, Markesich D, Payne R, Potter DG, Qian N, Shaw J, Schrick J, Shi ZZ, Sparks MJ, Van Sligtenhorst I, Vogel P, Walke W, Xu N, Zhu Q, Person C, Sands AT, Wnk1 kinase deficiency lowers blood pressure in mice: a gene-trap screen to identify potential targets for therapeutic intervention. Proc Natl Acad Sci U S A. 2003 Nov 25;100(24):14109-14

- Lyon MF, Rastan S, Brown SDM, in Lyon MF, Rastan S, Brown SDM (eds), Genetic Variants and Strains of the Laboratory Mouse. 3rd Edition. 1996Oxford: Oxford University Press.

- Juriloff DM, Sulik KK, Roderick TH, Hogan BK, Genetic and developmental studies of a new mouse mutation that produces otocephaly. J Craniofac Genet Dev Biol. 1985;5(2):121-45

- Roderick TH, Using inversions to detect and study recessive lethals and detrimentals in mice, in de Serres FJ, Sheridan W (eds), Utilization of Mammalian Specific Locus Studies in Hazard Evaluation and Estimation of Genetic Risk. 1983:135-67. Plenum Publishing Corp..

- Roderick TH, Hawes NL, Two radiation-induced chromosomal inversions in mice (Mus musculus). Proc Natl Acad Sci U S A. 1970 Oct;67(2):961-7

GPR70

Knockout references

http://www.mousephenotype.org/data/genes/MGI:1927505
http://www.informatics.jax.org/marker/MGI:1927505

Phenotype references

- van der Weyden L, Adams DJ, Cancer of mice and men: old twists and new tails. J Pathol. 2013 May;230(1):4-16

- Foster SR, Porrello ER, Purdue B, Chan HW, Voigt A, Frenzel S, Hannan RD, Moritz KM, Simmons DG, Molenaar P, Roura E, Boehm U, Meyerhof W, Thomas WG, Expression, regulation and putative nutrient-sensing function of taste GPCRs in the heart. PLoS One. 2013;8(5):e64579

- Simon BR, Parlee SD, Learman BS, Mori H, Scheller EL, Cawthorn WP, Ning X, Gallagher K, Tyrberg B, Assadi-Porter FM, Evans CR, MacDougald OA, Artificial sweeteners stimulate adipogenesis and suppress lipolysis independently of sweet taste receptors. J Biol Chem. 2013 Nov 8;288(45):32475-89

- Kusuhara Y, Yoshida R, Ohkuri T, Yasumatsu K, Voigt A, Hubner S, Maeda K, Boehm U, Meyerhof W, Ninomiya Y, Taste responses in mice lacking taste receptor subunit T1R1. J Physiol. 2013 Apr 1;591(Pt 7):1967-85

- Meyer D, Voigt A, Widmayer P, Borth H, Huebner S, Breit A, Marschall S, de Angelis MH, Boehm U, Meyerhof W, Gudermann T, Boekhoff I, Expression of Tas1 taste receptors in mammalian spermatozoa: functional role of Tas1r1 in regulating basal Ca(2)(+) and cAMP concentrations in spermatozoa. PLoS One. 2012;7(2):e32354

- Voigt A, Hubner S, Lossow K, Hermans-Borgmeyer I, Boehm U, Meyerhof W, Genetic labeling of Tas1r1 and Tas2r131 taste receptor cells in mice. Chem Senses. 2012 Nov;37(9):897-911

- Skarnes WC, Rosen B, West AP, Koutsourakis M, Bushell W, Iyer V, Mujica AO, Thomas M, Harrow J, Cox

GPR71

Knockout references

http://www.mousephenotype.org/data/genes/MGI:1933546
http://www.informatics.jax.org/marker/MGI:1933546

Phenotype references

- Smith KR, Spector AC, The importance of the presence of a 5'-ribonucleotide and the contribution of the T1R1 + T1R3 heterodimer and an additional low-affinity receptor in the taste detection of L-glutamate as assessed psychophysically. J Neurosci. 2014 Sep 24;34(39):13234-45

- Simon BR, Learman BS, Parlee SD, Scheller EL, Mori H, Cawthorn WP, Ning X, Krishnan V, Ma YL, Tyrberg B, MacDougald OA, Sweet taste receptor deficient mice have decreased adiposity and increased bone mass. PLoS One. 2014;9(1):e86454

- Simon BR, Parlee SD, Learman BS, Mori H, Scheller EL, Cawthorn WP, Ning X, Gallagher K, Tyrberg B, Assadi-Porter FM, Evans CR, MacDougald OA, Artificial sweeteners stimulate adipogenesis and suppress lipolysis independently of sweet taste receptors. J Biol Chem. 2013 Nov 8;288(45):32475-89

- Treesukosol Y, Spector AC, Orosensory detection of sucrose, maltose, and glucose is severely impaired in mice lacking T1R2 or T1R3, but Polycose sensitivity remains relatively normal. Am J Physiol Regul Integr Comp Physiol. 2012 Jul 15;303(2):R218-35

- Kyriazis GA, Soundarapandian MM, Tyrberg B, Sweet taste receptor signaling in beta cells mediates fructose-induced potentiation of glucose-stimulated insulin secretion. Proc Natl Acad Sci U S A. 2012 Feb 21;109(8):E524-32

- Geraedts MC, Takahashi T, Vigues S, Markwardt ML, Nkobena A, Cockerham RE, Hajnal A, Dotson CD, Rizzo MA, Munger SD, Transformation of postingestive glucose responses after deletion of sweet taste receptor subunits or gastric bypass surgery. Am J Physiol Endocrinol Metab. 2012 Aug;303(4):E464-74

- Treesukosol Y, Smith KR, Spector AC, Behavioral evidence for a glucose polymer taste receptor that is independent of the T1R2+3 heterodimer in a mouse model. J Neurosci. 2011 Sep 21;31(38):13527-34

- Skarnes WC, Rosen B, West AP, Koutsourakis M, Bushell W, Iyer V, Mujica AO, Thomas M, Harrow J, Cox T, Jackson D, Severin J, Biggs P, Fu J, Nefedov M, de Jong PJ, Stewart AF, Bradley A, A conditional knockout resource for the genome-wide study of mouse gene function. Nature. 2011 Jun 16;474(7351):337- 42

- Feng J, Petersen CD, Coy DH, Jiang JK, Thomas CJ, Pollak MR, Wank SA, Calcium-sensing receptor is a physiologic multimodal chemosensor regulating gastric G-cell growth and gastrin secretion. Proc Natl Acad Sci U S A. 2010 Oct 12;107(41):17791-6

- Iwatsuki K, Nomura M, Sh bata A, Ichikawa R, Enciso PL, Wang L, Takayanagi R, Torii K, Uneyama H, Generation and characterization of T1R2-LacZ knock-in mouse. Biochem Biophys Res Commun. 2010 Nov 19;402(3):495-9

- Treesukosol Y, Blonde GD, Spector AC, T1R2 and T1R3 subunits are individually unnecessary for normal affective licking responses to polycose: implications for saccharide taste receptors in mice. Am J Physiol Regul Integr Comp Physiol. 2009 Apr;296(4):R855-65

- Zhao GQ, Zhang Y, Hoon MA, Chandrashekar J, Erlenbach I, Ryba NJ, Zuker CS, The receptors for mammalian sweet and umami taste. Cell. 2003 Oct 31;115(3):255-66

- Nishijima I, Mills A, Qi Y, Mills M, Bradley A, Two new balancer chromosomes on mouse chromosome 4 to facilitate functional annotation of human chromosome 1p. Genesis. 2003 Jul;36(3):142-8

- Lyon MF, Rastan S, Brown SDM, in Lyon MF, Rastan S, Brown SDM (eds), Genetic Variants and Strains of the Laboratory Mouse. 3rd Edition. 1996Oxford: Oxford University Press.

GPR73

Knockout references

http://www.mousephenotype.org/data/genes/MGI:1929676

http://www.informatics.jax.org/marker/MGI:1929676

Phenotype references

- Szatkowski C, Vallet J, Dormishian M, Messaddeq N, Valet P, Boulberdaa M, Metzger D, Chambon P, Nebigil CG, Prokineticin receptor 1 as a novel suppressor of preadipocyte proliferation and differentiation to control obesity. PLoS One. 2013;8(12):e81175

- Bou berdaa M, Turkeri G, Urayama K, Dormishian M, Szatkowski C, Zimmer L, Messaddeq N, Laugel V, Dolle P, Nebigil CG, Genetic inactivation of prokineticin receptor-1 leads to heart and kidney disorders. Arterioscler Thromb Vasc Biol. 2011 Apr;31(4):842-50

- Giannini E, Lattanzi R, Nicotra A, Campese AF, Grazioli P, Screpanti I, Ba boni G, Salvadori S, Sacerdote P, Negri L, The chemokine Bv8/prokineticin 2 is up-regulated in inflammatory granulocytes and modulates inflammatory pain. Proc Natl Acad Sci U S A. 2009 Aug 25;106(34):14646-51

- Urayama K, Guilini C, Turkeri G, Takir S, Kurose H, Messaddeq N, Dierich A, Nebigil CG, Prokineticin receptor-1 induces neovascularization and epicardial-derived progenitor cell differentiation. Arterioscler Thromb Vasc Biol. 2008 May;28(5):841-9

- Matsumoto S, Yamazaki C, Masumoto KH, Nagano M, Naito M, Soga T, Hiyama H, Matsumoto M, Takasaki J, Kamohara M, Matsuo A, Ishii H, Kobori M, Katoh M, Matsushime H, Furuichi K, Shigeyoshi Y, Abnormal development of the olfactory bulb and reproductive system in mice lacking prokineticin receptor PKR2. Proc Natl Acad Sci U SA. 2006 Mar 14;103(11):4140-5

- Negri L, Lattanzi R, Giannini E, Colucci M, Margheriti F, Melchiorri P, Vellani V, Tian H, De Felice M, Porreca F, Impaired nociception and inflammatory pain sensation in mice lacking the prokineticin receptor PKR1: focus on interaction between PKR1 and the capsaicin receptor TRPV1 in pain behavior. J Neurosci. 2006 Jun 21;26(25):6716-27

- Akeson EC, Donahue LR, Beamer WG, Shultz KL, Ackert-Bicknell C, Rosen CJ, Corrigan J, Davisson MT, Chromosomal inversion discovered in C3H/HeJ mice. Genomics. 2006 Feb;87(2):311-3

- Martucci C, Franchi S, Giannini E, Tian H, Melchiorri P, Negri L, Sacerdote P, Bv8, the amphibian homologue of the mammalian prokineticins, induces a proinflammatory phenotype of mouse macrophages. Br J Pharmacol. 2006 Jan;147(2):225-34

- Akeson EC, Chromosomal inversion discovered in C3H/HeJ mice. JAX Notes. 2003;491:15

GPR73l1

Knockout references

http://www.mousephenotype.org/data/genes/MGI:2181363
http://www.informatics.jax.org/marker/MGI:2181363

Phenotype references

- Xiao L, Zhang C, Li X, Gong S, Hu R, Balasubramanian R, Crowley W WF Jr, Hastings MH, Zhou QY, Signaling role of prokineticin 2 on the estrous cycle of female mice. PLoS One. 2014;9(3):e90860
- Jethwa PH, I'Anson H, Warner A, Prosser HM, Hastings MH, Maywood ES, Ebling FJ, Loss of prokineticin receptor 2 signaling predisposes mice to torpor. Am J Physiol Regul Integr Comp Physiol. 2008 Jun;294(6):R1968-79
- Prosser HM, Bradley A, Caldwell MA, Olfactory bulb hypoplasia in Prokr2 null mice stems from defective neuronal progenitor migration and differentiation. Eur J Neurosci. 2007 Dec;26(12):3339-44
- Prosser HM, Bradley A, Chesham JE, Ebling FJ, Hastings MH, Maywood ES, Prokineticin receptor 2 (Prokr2) is essential for the regulation of circadian behavior by the suprachiasmatic nuclei. Proc Natl Acad Sci U S A. 2007 Jan 9;104(2):648-53
- Matsumoto S, Yamazaki C, Masumoto KH, Nagano M, Naito M, Soga T, Hiyama H, Matsumoto M, Takasaki J, Kamohara M, Matsuo A, Ishii H, Kobori M, Katoh M, Matsushime H, Furuichi K, Shigeyoshi Y, Abnormal development of the olfactory bulb and reproductive system in mice lacking prokineticin receptor PKR2. Proc Natl Acad Sci U SA. 2006 Mar 14;103(11):4140-5
- Lyon MF, Rastan S, Brown SDM, in Lyon MF, Rastan S, Brown SDM (eds), Genetic Variants and Strains of the Laboratory Mouse. 3rd Edition. 1996Oxford: Oxford University Press.
- Roderick TH, Using inversions to detect and study recessive lethals and detrimentals in mice, in de Serres FJ, Sheridan W (eds), Utilization of Mammalian Specific Locus Studies in Hazard Evaluation and Estimation of Genetic Risk. 1983:135-67. Plenum Publishing Corp..
- Roderick TH, Hawes NL, Nineteen paracentric chromosomal inversions in mice. Genetics. 1974 Jan;76(1):109-17

GPR74

Knockout references

http://www.mousephenotype.org/data/genes/MGI:1860130
http://www.informatics.jax.org/marker/MGI:1860130

Phenotype references

- Lyon MF, Rastan S, Brown SDM, in Lyon MF, Rastan S, Brown SDM (eds), Genetic Variants and Strains of the Laboratory Mouse. 3rd Edition. 1996Oxford: Oxford University Press.
- Beechey CV, List of Chromosome Anomalies. Mouse Genome. 1994;92:336-47
- Roderick TH, Using inversions to detect and study recessive lethals and detrimentals in mice, in de Serres FJ, Sheridan W (eds), Utilization of Mammalian Specific Locus Studies in Hazard Evaluation and Estimation of Genetic Risk. 1983:135-67. Plenum Publishing Corp..
- Roderick TH, Hawes NL, Nineteen paracentric chromosomal inversions in mice. Genetics. 1974 Jan;76(1):109-17

- Roderick TH, Producing and detecting paracentric chromosomal inversions in mice. Mutat Res. 1971 Jan;11(1):59-69

- Roderick TH, Hawes NL, Two radiation-induced chromosomal inversions in mice (Mus musculus). Proc Natl Acad Sci U S A. 1970 Oct;67(2):961-7

GPR77

Knockout references

http://www.mousephenotype.org/data/genes/MGI:2442013
http://www.informatics.jax.org/marker/MGI:2442013

Phenotype references

- Kim H, Erdman LK, Lu Z, Serghides L, Zhong K, Dhabangi A, Musoke C, Gerard C, Cserti-Gazdewich C, Liles WC, Kain KC, Functional roles for C5a and C5aR but not C5L2 in the pathogenesis of human and experimental cerebral malaria. Infect Immun. 2014 Jan;82(1):371-9

- Vijayan S, Asare Y, Grommes J, Soehnlein O, Lutgens E, Shagdarsuren G, Togtokh A, Jacobs MJ, Fischer JW, Bernhagen J, Weber C, Schober A, Shagdarsuren E, High Expression of C5L2 Correlates with High Proinflammatory Cytokine Expression in Advanced Human Atherosclerotic Plaques. Am J Pathol. 2014 Jul;184(7):2123-33

- Roy C, Paglialunga S, Schaart G, Moonen-Kornips E, Meex RC, Phielix E, Hoeks J, Hesselink MK, Cianflone K, Schrauwen P, Relationship of C5L2 receptor to skeletal muscle substrate utilization. PLoS One. 2013;8(2):e57494

- Gauvreau D, Gupta A, Fisette A, Tom FQ, Cianflone K, Deficiency of C5L2 increases macrophage infiltration and alters adipose tissue function in mice. PLoS One. 2013;8(4):e60795

- Wang R, Lu B, Gerard C, Gerard NP, Disruption of the complement anaphylatoxin receptor C5L2 exacerbates inflammation in allergic contact dermatitis. J Immunol. 2013 Oct 15;191(8):4001-9

- Bosmann M, Haggadone MD, Zetoune FS, Sarma JV, Ward PA, The interaction between C5a and both C5aR and C5L2 receptors is required for production of G-CSF during acute inflammation. Eur J Immunol. 2013 Jul;43(7):1907-13

- Raby AC, Holst B, Davies J, Colmont C, Laumonnier Y, Coles B, Shah S, Hall J, Topley N, Kohl J, Morgan BP, Labeta MO, TLR activation enhances C5a-induced pro-inflammatory responses by negatively modulating the second C5a receptor, C5L2. Eur J Immunol. 2011 Sep;41(9):2741-52

- Fusakio ME, Mohammed JP, Laumonnier Y, Hoebe K, Kohl J, Mattner J, C5a regulates NKT and NK cell functions in sepsis. J Immunol. 2011 Dec 1;187(11):5805-12

- Skarnes WC, Rosen B, West AP, Koutsourakis M, Bushell W, Iyer V, Mujica AO, Thomas M, Harrow J, Cox T, Jackson D, Severin J, Biggs P, Fu J, Nefedov M, de Jong PJ, Stewart AF, Bradley A, A conditional knockout resource for the genome-wide study of mouse gene function. Nature. 2011 Jun 16;474(7351):337- 42

- Zhang X, Schmudde I, Laumonnier Y, Pandey MK, Clark JR, Konig P, Gerard NP, Gerard C, Wills-Karp M, Kohl J, A critical role for C5L2 in the pathogenesis of experimental allergic asthma. J Immunol. 2010 Dec 1;185(11):6741-52

- He S, Atkinson C, Qiao F, Cianflone K, Chen X, Tomlinson S, A complement-dependent balance between hepatic ischemia/reperfusion injury and liver regeneration in mice. J Clin Invest. 2009 Aug;119(8):2304-16

- Rittirsch D, Flierl MA, Nadeau BA, Day DE, Huber-Lang M, Mackay CR, Zetoune FS, Gerard NP, Cianflone K, Kohl J, Gerard C, Sarma JV, Ward PA, Functional roles for C5a receptors in sepsis. Nat Med. 2008 May;14(5):551-7

- Paglialunga S, Schrauwen P, Roy C, Moonen-Kornips E, Lu H, Hesselink MK, Deshaies Y, Richard D, Cianflone K, Reduced adipose tissue triglyceride synthesis and increased muscle fatty acid oxidation in C5L2 knockout mice. J Endocrinol. 2007 Aug;194(2):293-304

- Gerard NP, Lu B, Liu P, Craig S, Fujiwara Y, Okinaga S, Gerard C, An anti-inflammatory function for the complement anaphylatoxin C5a-binding protein, C5L2. J Biol Chem. 2005 Dec 2;280(48):39677-80

- Valenzuela DM, Murphy AJ, Frendewey D, Gale NW, Economides AN, Auerbach W, Poueymirou WT, Adams NC, Rojas J, Yasenchak J, Chernomorsky R, Boucher M, Elsasser AL, Esau L, Zheng J, Griffiths JA, Wang X, Su H, Xue Y, Dominguez MG, Noguera I, Torres R, Macdonald LE, Stewart AF, DeChiara TM, Yancopoulos GD, High-throughput engineering of the mouse genome coupled with high-resolution expression analysis. Nat Biotechnol. 2003Jun;21(6):652-9

GPR80

Knockout references

http://www.mousephenotype.org/data/genes/MGI:2685145
http://www.informatics.jax.org/marker/MGI:2685145

Phenotype references

- Kanaoka Y, Maekawa A, Austen KF, Identification of GPR99 protein as a potential third cysteinyl leukotriene receptor with a preference for leukotriene E4 ligand. J Biol Chem. 2013 Apr 19;288(16):10967-72

- Tokonami N, Morla L, Centeno G, Mordasini D, Ramakrishnan SK, Nikolaeva S, Wagner CA, Bonny O, Houillier P, Doucet A, Firsov D, alpha-Ketoglutarate regulates acid-base balance through an intrarenal paracrine mechanism. J Clin Invest. 2013 Jul 1;123(7):3166-71

- Cummings HE, Liu T, Feng C, Laidlaw TM, Conley PB, Kanaoka Y, Boyce JA, Cutting edge: Leukotriene C4 activates mouse platelets in plasma exclusively through the type 2 cysteinyl leukotriene receptor. J Immunol. 2013 Dec 15;191(12):5807-10

- Zambrowicz BP, Abuin A, Ramirez-Solis R, Richter LJ, Piggott J, BeltrandelRio H, Buxton EC, Edwards J, Finch RA, Friddle CJ, Gupta A, Hansen G, Hu Y, Huang W, Jaing C, Key BW Jr, Kipp P, Kohlhauff B, Ma ZQ, Markesich D, Payne R, Potter DG, Qian N, Shaw J, Schrick J, Shi ZZ, Sparks MJ, Van Sligtenhorst I, Vogel P, Walke W, Xu N, Zhu Q, Person C, Sands AT, Wnk1 kinase deficiency lowers blood pressure in mice: a gene-trap screen to identify potential targets for therapeutic intervention. Proc Natl Acad Sci U S A. 2003 Nov 25;100(24):14109-14

- Roix J, Hagge-Greenberg A, Bissonnette D, Rodick S, Russell L, O'Brien T, Molecular and functional mapping of the piebald deletion complex on mouse chromosome 14. Genetics. 2001 Feb;157(2):803-15

- Lyon MF, Rastan S, Brown SDM, in Lyon MF, Rastan S, Brown SDM (eds), Genetic Variants and Strains of the Laboratory Mouse. 3rd Edition. 1996Oxford: Oxford University Press.

- Roderick TH, Using inversions to detect and study recessive lethals and detrimentals in mice, in de Serres FJ, Sheridan W (eds), Utilization of Mammalian Specific Locus Studies in Hazard Evaluation and Estimation of Genetic Risk. 1983:135-67. Plenum Publishing Corp.

GPR81

Knockout references

http://www.mousephenotype.org/data/genes/MGI:2441671
http://www.informatics.jax.org/marker/MGI:2441671

Phenotype references

- Ahmed K, Tunaru S, Tang C, Muller M, Gille A, Sassmann A, Hanson J, Offermanns S, An autocrine lactate loop mediates insulin-dependent inh bition of lipolysis through GPR81. Cell Metab. 2010 Apr 7;11(4):311-9

- Liu C, Wu J, Zhu J, Kuei C, Yu J, Shelton J, Sutton SW, Li X, Yun SJ, Mirzadegan T, Mazur C, Kamme F, Lovenberg TW, Lactate inhibits lipolysis in fat cells through activation of an orphan G-protein-coupled receptor, GPR81. J Biol Chem. 2009 Jan 30;284(5):2811-22

- Cai TQ, Ren N, Jin L, Cheng K, Kash S, Chen R, Wright SD, Taggart AK, Waters MG, Role of GPR81 in lactate-mediated reduction of adipose lipolysis. Biochem Biophys Res Commun. 2008 Dec 19;377(3):987-91

- Lyon MF, Rastan S, Brown SDM, in Lyon MF, Rastan S, Brown SDM (eds), Genetic Variants and Strains of the Laboratory Mouse. 3rd Edition. 1996Oxford: Oxford University Press.

- Roderick TH, Using inversions to detect and study recessive lethals and detrimentals in mice, in de Serres FJ, Sheridan W (eds), Utilization of Mammalian Specific Locus Studies in Hazard Evaluation and Estimation of Genetic Risk. 1983:135-67. Plenum Publishing Corp..

- Roderick TH, Hawes NL, Nineteen paracentric chromosomal inversions in mice. Genetics. 1974 Jan;76(1):109-17

- Roderick TH, Producing and detecting paracentric chromosomal inversions in mice. Mutat Res. 1971 Jan;11(1):59-69

- Roderick TH, Hawes NL, Two radiation-induced chromosomal inversions in mice (Mus musculus). Proc Natl Acad Sci U S A. 1970 Oct;67(2):961-7

GPR83

Knockout references

http://www.mousephenotype.org/data/genes/MGI:95712
http://www.informatics.jax.org/marker/MGI:95712

Phenotype references

- E Vollmer L, Ghosal S, A Rush J, R Sallee F, P Herman J, Weinert M, Sah R, Attenuated stress-evoked anxiety, increased sucrose preference and delayed spatial learning in glucocorticoid-induced receptor- deficient mice. Genes Brain Behav. 2013 Mar;12(2):241-9

- Muller TD, Muller A, Yi CX, Habegger KM, Meyer CW, Gaylinn BD, Finan B, Heppner K, Trivedi C, Bielohuby M, Abplanalp W, Meyer F, Piechowski CL, Pratzka J, Stemmer K, Holland J, Hembree J, Bhardwaj N, Raver C, Ottaway N, Krishna R, Sah R, Sallee FR, WoodsSC, Perez-Tilve D, Bidlingmaier M, Thorner MO, Krude H, Smiley D, DiMarchi R, Hofmann S, Pfluger PT, Kleinau G, Biebermann H, Tschop MH, The orphan receptor Gpr83 regulates systemic energy metabolism via ghrelin-dependent and ghrelin-independent mechanisms. Nat Commun. 2013;4:1968

- Skarnes WC, Rosen B, West AP, Koutsourakis M, Bushell W, Iyer V, Mujica AO, Thomas M, Harrow J, Cox T, Jackson D, Severin J, Biggs P, Fu J, Nefedov M, de Jong PJ, Stewart AF, Bradley A, A conditional knockout resource for the genome-wide study of mouse gene function. Nature. 2011 Jun 16;474(7351):337- 42

- Toms C, Jessup H, Thompson C, Baban D, Davies K, Powrie F, Gpr83 expression is not required for the maintenance of intestinal immune homeostasis and regulation of T-cell-dependent colitis. Immunology. 2008 Nov;125(3):302-12

- Lu LF, Gavin MA, Rasmussen JP, Rudensky AY, G protein-coupled receptor 83 is dispensable for the development and function of regulatory T cells. Mol Cell Biol. 2007 Dec;27(23):8065-72

GPR84

Knockout references

http://www.mousephenotype.org/data/genes/MGI:1934129
http://www.informatics.jax.org/marker/MGI:1934129

Phenotype references

- Venkataraman C, Kuo F, The G-protein coupled receptor, GPR84 regulates IL-4 production by T lymphocytes in response to CD3 crosslinking. Immunol Lett. 2005 Nov 15;101(2):144-53

GPR85

Knockout references

http://www.mousephenotype.org/data/genes/MGI:1927851
http://www.informatics.jax.org/marker/MGI:1927851

Phenotype references

- Chen Q, Kogan JH, Gross AK, Zhou Y, Walton NM, Shin R, Heusner CL, Miyake S, Tajinda K, Tamura K, Matsumoto M, SREB2/GPR85, a schizophrenia risk factor, negatively regulates hippocampal adult neurogenesis and neurogenesis-dependent learning and memory. Eur J Neurosci. 2012 Sep;36(5):2597-608

- Skarnes WC, Rosen B, West AP, Koutsourakis M, Bushell W, Iyer V, Mujica AO, Thomas M, Harrow J, Cox T, Jackson D, Severin J, Biggs P, Fu J, Nefedov M, de Jong PJ, Stewart AF, Bradley A, A conditional knockout resource for the genome-wide study of mouse gene function. Nature. 2011 Jun 16;474(7351):337- 42

- Matsumoto M, Straub RE, Marenco S, Nicodemus KK, Matsumoto S, Fujikawa A, Miyoshi S, Shobo M, Takahashi S, Yarimizu J, Yuri M, Hiramoto M, Morita S, Yokota H, Sasayama T, Terai K, Yoshino M, Miyake A, Callicott JH, Egan MF, Meyer-Lindenberg A, Kempf L, Honea R, Vakkalanka RK, Takasaki J, Kamohara M, Soga T, Hiyama H, Ishii H, Matsuo A, Nishimura S, Matsuoka N, Kobori M, Matsushime H, Katoh M, Furuichi K, Weinberger DR, The evolutionarily conserved G protein-coupled receptor SREB2/GPR85 influences brain size, behavior, and vulnerability to schizophrenia. Proc Natl Acad Sci U S A. 2008 Apr 22;105(16):6133-8

GPR86

Knockout references

http://www.mousephenotype.org/data/genes/MGI:1921441
http://www.informatics.jax.org/marker/MGI:1921441

Phenotype references

- Wang N, Robaye B, Gossiel F, Boeynaems JM, Gartland A, The P2Y13 receptor regulates phosphate metabolism and FGF-23 secretion with effects on skeletal development. FASEB J. 2014 May;28(5):2249-59

- Ben Addi A, Cammarata D, Conley PB, Boeynaems JM, Robaye B, Role of the P2Y12 receptor in the modulation of murine dendritic cell function by ADP. J Immunol. 2010 Nov 15;185(10):5900-6

- Yamin TT, Champy MF, Selloum M, Bedu E, Carballo-Jane E, Gerckens L, Luell S, Meurer R, Chin J, Mudgett J, Puig O, Altered lipoprotein metabolism in P2Y(13) knockout mice. Biochim Biophys Acta. 2010 Dec;1801(12):1349-60

- Fabre AC, Malaval C, Ben Addi A, Verdier C, Pons V, Serhan N, Lichtenstein L, Combes G, Huby T, Briand F, Collet X, Nijstad N, Tietge UJ, Robaye B, Perret B, Boeynaems JM, Martinez LO, P2Y13 receptor is critical for reverse cholesterol transport. Hepatology. 2010 Oct;52(4):1477-83

- Cook SA, Akeson EC, Calvano C, Johnson KR, Mandell J, Hawes NL, Bronson RT, Roderick TH, Davisson MT, Mouse paracentric inversion In(3)55Rk mutates the urate oxidase gene. Cytogenet Cell Genet. 2001;93(1-2):77-82

GPR87

Knockout references

http://www.mousephenotype.org/data/genes/MGI:1934133;
http://www.informatics.jax.org/allele/MGI:5428728?page=alleleDetail&id=MGI:5428728

Phenotype references

- Cook SA, Akeson EC, Calvano C, Johnson KR, Mandell J, Hawes NL, Bronson RT, Roderick TH, Davisson MT, Mouse paracentric inversion In(3)55Rk mutates the urate oxidase gene. Cytogenet Cell Genet. 2001;93(1-2):77-82

GPR88

Knockout references

http://www.mousephenotype.org/data/genes/MGI:1927653

Phenotype references

- Zhang K, Chammas C, Soghomonian JJ, Loss of glutamic acid decarboxylase (Gad67) in striatal neurons expressing the Drdr1a dopamine receptor prevents L-DOPA-induced dyskinesia in 6-hydroxydopamine-lesioned mice. Neuroscience. 2015 Sep 10;303:586-94

- Zhang K, Hill K, Labak S, Blatt GJ, Soghomonian JJ, Loss of glutamic acid decarboxylase (Gad67) in Gpr88-expressing neurons induces learning and social behavior deficits in mice. Neuroscience. 2014 Sep 5;275:238-47

- Quintana A, Sanz E, Wang W, Storey GP, Guler AD, Wanat MJ, Roller BA, La Torre A, Amieux PS, McKnight GS, Bamford NS, Palmiter RD, Lack of GPR88 enhances medium spiny neuron activity and alters motor- and cue-dependent behaviors. Nat Neurosci. 2012 Nov;15(11):1547-55

- Beutler LR, Wanat MJ, Quintana A, Sanz E, Bamford NS, Zweifel LS, Palmiter RD, Balanced NMDA receptor activity in dopamine D1 receptor (D1R)- and D2R-expressing medium spiny neurons is required for amphetamine sensitization. Proc Natl Acad Sci U S A. 2011 Mar 8;108(10):4206-11

- Parker JG, Beutler LR, Palmiter RD, The Contribution of NMDA Receptor Signaling in the Corticobasal Ganglia Reward Network to Appetitive Pavlovian Learning. J Neurosci. 2011 Aug 3;31(31):11362-9

- Cook SA, Akeson EC, Calvano C, Johnson KR, Mandell J, Hawes NL, Bronson RT, Roderick TH, Davisson MT, Mouse paracentric inversion In(3)55Rk mutates the urate oxidase gene. Cytogenet Cell Genet. 2001;93(1-2):77-82

- Lyon MF, Rastan S, Brown SDM, in Lyon MF, Rastan S, Brown SDM (eds), Genetic Variants and Strains of the Laboratory Mouse. 3rd Edition. 1996Oxford: Oxford University Press.

- Roderick TH, Using inversions to detect and study recessive lethals and detrimentals in mice, in de Serres FJ, Sheridan W (eds), Utilization of Mammalian Specific Locus Studies in Hazard Evaluation and Estimation of Genetic Risk. 1983:135-67. Plenum Publishing Corp..

GPR91

Knockout references

http://www.mousephenotype.org/data/genes/MGI:1934135+F88http://www.mousephenotype.org/data/genes/MGI:1934135
http://www.informatics.jax.org/marker/MGI:1934135

Phenotype references

Favret S, Binet F, Lapalme E, Leboeuf D, Carbadillo J, Rubic T, Picard E, Mawambo G, Tetreault N, Joyal JS, Chemtob S, Sennlaub F, Sangiovanni JP, Guimond M, Sapieha P, Deficiency in the metabolite receptor SUCNR1 (GPR91) leads to outer retinal lesions. Aging (Albany NY). 2013 Jun;5(6):427-44

Skarnes WC, Rosen B, West AP, Koutsourakis M, Bushell W, Iyer V, Mujica AO, Thomas M, Harrow J, Cox T, Jackson D, Severin J, Biggs P, Fu J, Nefedov M, de Jong PJ, Stewart AF, Bradley A, A conditional knockout resource for the genome-wide study of mouse gene function. Nature. 2011 Jun 16;474(7351):337-42

Toma I, Kang JJ, Sipos A, Vargas S, Bansal E, Hanner F, Meer E, Peti-Peterdi J, Succinate receptor GPR91 provides a direct link between high glucose levels and renin release in murine and rabbit kidney. J Clin Invest. 2008 Jul;118(7):2526-34

Rubic T, Lametschwandtner G, Jost S, Hinteregger S, Kund J, Carballido-Perrig N, Schwarzler C, Junt T, Voshol H, Meingassner JG, Mao X, Werner G, Rot A, Carballido JM, Triggering the succinate receptor GPR91 on dendritic cells enhances immunity. Nat Immunol. 2008 Nov;9(11):1261-9

Cook SA, Akeson EC, Calvano C, Johnson KR, Mandell J, Hawes NL, Bronson RT, Roderick TH, Davisson MT, Mouse paracentric inversion In(3)55Rk mutates the urate oxidase gene. Cytogenet Cell Genet. 2001;93(1-2):77-82

GPR93

Knockout references

http://www.mousephenotype.org/data/genes/MGI:2685918
http://www.informatics.jax.org/marker/MGI:2685918

Phenotype references

- Lin ME, Rivera RR, Chun J, Targeted deletion of LPA5 identifies novel roles for lysophosphatidic acid signaling in development of neuropathic pain. J Biol Chem. 2012 May 18;287(21):17608-17

- Callaerts-Vegh Z, Leo S, Vermaercke B, Meert T, D'Hooge R, LPA(5) receptor plays a role in pain sensitivity, emotional exploration and reversal learning. Genes Brain Behav. 2012 Oct 8;

- Skarnes WC, Rosen B, West AP, Koutsourakis M, Bushell W, Iyer V, Mujica AO, Thomas M, Harrow J, Cox T, Jackson D, Severin J, Biggs P, Fu J, Nefedov M, de Jong PJ, Stewart AF, Bradley A, A conditional knockout resource for the genome-wide study of mouse gene function. Nature. 2011 Jun 16;474(7351):337- 42

- Hansen GM, Markesich DC, Burnett MB, Zhu Q, Dionne KM, Richter LJ, Finnell RH, Sands AT, Zambrowicz BP, Abuin A, Large-scale gene trapping in C57BL/6N mouse embryonic stem cells. Genome Res. 2008 Oct;18(10):1670-9

- Taniwaki T, Haruna K, Nakamura H, Sekimoto T, Oike Y, Imaizumi T, Saito F, Muta M, Soejima Y, Utoh A, Nakagata N, Araki M, Yamamura K, Araki K, Characterization of an exchangeable gene trap using pU-17 carrying a stop codon-beta geo cassette. Dev Growth Differ. 2005 Apr;47(3):163-72

- Zambrowicz BP, Abuin A, Ramirez-Solis R, Richter LJ, Piggott J, BeltrandelRio H, Buxton EC, Edwards J, Finch RA, Friddle CJ, Gupta A, Hansen G, Hu Y, Huang W, Jaing C, Key BW Jr, Kipp P, Kohlhauff B, Ma ZQ, Markesich D, Payne R, Potter DG, Qian N, Shaw J, Schrick J, Shi ZZ, Sparks MJ, Van Sligtenhorst I, Vogel P, Walke W, Xu N, Zhu Q, Person C, Sands AT, Wnk1 kinase deficiency lowers blood pressure in mice: a gene-trap screen to identify potential targets for therapeutic intervention. Proc Natl Acad Sci U S A. 2003 Nov 25;100(24):14109-14

- Araki K, Imaizumi T, Sekimoto T, Yoshinobu K, Yoshimuta J, Akizuki M, Miura K, Araki M, Yamamura K, Exchangeable gene trap using the Cre/mutated lox system. Cell Mol Biol (Noisy-le-grand). 1999 Jul;45(5):737-50

GPR98

Knockout references

http://www.mousephenotype.org/data/genes/MGI:1274784
http://www.informatics.jax.org/marker/MGI:1274784

Phenotype references

- Zou J, Zheng T, Ren C, Askew C, Liu XP, Pan B, Holt JR, Wang Y, Yang J, Deletion of PDZD7 disrupts the Usher syndrome type 2 protein complex in cochlear hair cells and causes hearing loss in mice. Hum Mol Genet. 2014 May 1;23(9):2374-90

- Cosgrove D, Zallocchi M, Usher protein functions in hair cells and photoreceptors. Int J Biochem Cell Biol. 2014 Jan;46:80-9

- Shin D, Lin ST, Fu YH, Ptacek LJ, Very large G protein-coupled receptor 1 regulates myelin-associated glycoprotein via Galphas/Galphaq-mediated protein kinases A/C. Proc Natl Acad Sci U S A. 2013 Nov 19;110(47):19101-6

- Goodyear RJ, Jones SM, Sharifi L, Forge A, Richardson GP, Hair bundle defects and loss of function in the vestibular end organs of mice lacking the receptor-like inositol lipid phosphatase PTPRQ. J Neurosci. 2012 Feb 22;32(8):2762-72

- Zou J, Luo L, Shen Z, Chiodo VA, Ambati BK, Hauswirth WW, Yang J, Whirlin Replacement Restores the Formation of the USH2 Protein Complex in Whirlin Knockout Photoreceptors. Invest Ophthalmol Vis Sci. 2011 Apr;52(5):2343-51

- Yagi H, Noguchi Y, Kitamura K, Sato M, Deficiency of Vlgr1 resulted in deafness and suscept bility to audiogenic seizures while the degree of hearing impairment was not correlated with seizure severity in C57BL/6- and 129-backcrossed lines of Vlgr1 knockout mice. Neurosci Lett. 2009 Sep 18;461(2):190-5

- Maerker T, van Wijk E, Overlack N, Kersten FF, McGee J, Goldmann T, Sehn E, Roepman R, Walsh EJ, Kremer H, Wolfrum U, A novel Usher protein network at the periciliary reloading point between molecular transport machineries in vertebrate photoreceptor cells. Hum Mol Genet. 2008 Jan 1;17(1):71-86

- Yagi H, Tokano H, Maeda M, Takabayashi T, Nagano T, Kiyama H, Fujieda S, Kitamura K, Sato M, Vlgr1 is required for proper stereocilia maturation of cochlear hair cells. Genes Cells. 2007 Feb;12(2):235-50

- Schwander M, Sczaniecka A, Grillet N, Bailey JS, Avenarius M, Najmabadi H, Steffy BM, Federe GC, Lagler EA, Banan R, Hice R, Grabowski-Boase L, Keithley EM, Ryan AF, Housley GD, Wiltshire T, Smith RJ, Tarantino LM, Muller U, A forward genetics screen in mice identifies recessive deafness traits and reveals that pejvakin is essential for outer hair cell function. J Neurosci. 2007 Feb 28;27(9):2163-75

- Michalski N, Michel V, Bahloul A, Lefevre G, Barral J, Yagi H, Chardenoux S, Weil D, Martin P, Hardelin JP, Sato M, Petit C, Molecular characterization of the ankle-link complex in cochlear hair cells and its role in the hair bundle functioning. J Neurosci. 2007 Jun 13;27(24):6478-88

- Johnson KR, Zheng QY, Noben-Trauth K, Strain background effects and genetic modifiers of hearing in mice. Brain Res. 2006 May 26;1091(1):79-88

- McGee J, Goodyear RJ, McMillan DR, Stauffer EA, Holt JR, Locke KG, Birch DG, Legan PK, White PC, Walsh EJ, Richardson GP, The very large G-protein-coupled receptor VLGR1: a component of the ankle link complex required for the normal development of auditory hair bundles. J Neurosci. 2006 Jun 14;26(24):6543- 53

GPR100

Knockout references

http://www.mousephenotype.org/data/genes/MGI:2182926
http://www.informatics.jax.org/marker/MGI:2182926

Phenotype references

- Grosse J, Heffron H, Burling K, Akhter Hossain M, Habib AM, Rogers GJ, Richards P, Larder R, Rimmington D, Adriaenssens AA, Parton L, Powell J, Binda M, Colledge WH, Doran J, Toyoda Y, Wade JD, Aparicio S, Carlton MB, Coll AP, Reimann F, O'Rahilly S, Gribble FM, Insulin-like peptide 5 is an orexigenic gastrointestinal hormone. Proc Natl Acad Sci U S A. 2014 Jul 29;111(30):11133-8

- Cook SA, Akeson EC, Calvano C, Johnson KR, Mandell J, Hawes NL, Bronson RT, Roderick TH, Davisson MT, Mouse paracentric inversion In(3)55Rk mutates the urate oxidase gene. Cytogenet Cell Genet. 2001;93(1-2):77-82

- Lyon MF, Rastan S, Brown SDM, in Lyon MF, Rastan S, Brown SDM (eds), Genetic Variants and Strains of the Laboratory Mouse. 3rd Edition. 1996Oxford: Oxford University Press.

- Roderick TH, Using inversions to detect and study recessive lethals and detrimentals in mice, in de Serres FJ, Sheridan W (eds), Utilization of Mammalian Specific Locus Studies in Hazard Evaluation and Estimation of Genetic Risk. 1983:135-67. Plenum Publishing Corp..

GPR103

Knockout references

http://www.mousephenotype.org/data/genes/MGI:2677633
http://www.informatics.jax.org/marker/MGI:2677633

Phenotype references

- Skarnes WC, Rosen B, West AP, Koutsourakis M, Bushell W, Iyer V, Mujica AO, Thomas M, Harrow J, Cox T, Jackson D, Severin J, Biggs P, Fu J, Nefedov M, de Jong PJ, Stewart AF, Bradley A, A conditional knockout resource for the genome-wide study of mouse gene function. Nature. 2011 Jun 16;474(7351):337- 42

- Baribault H, Danao J, Gupte J, Yang L, Sun B, Richards W, Tian H, The G-protein-coupled receptor GPR103 regulates bone formation. Mol Cell Biol. 2006 Jan;26(2):709-17

- Cook SA, Akeson EC, Calvano C, Johnson KR, Mandell J, Hawes NL, Bronson RT, Roderick TH, Davisson MT, Mouse paracentric inversion In(3)55Rk mutates the urate oxidase gene. Cytogenet Cell Genet. 2001;93(1-2):77-82

GPR106

Knockout references

http://www.mousephenotype.org/data/genes/MGI:2153463
http://www.informatics.jax.org/marker/MGI:2153463

Phenotype references

- Kaftanovskaya EM, Lopez C, Ferguson L, Myhr C, Agoulnik AI, Genetic ablation of androgen receptor signaling in fetal Leydig cell lineage affects Leydig cell functions in adult testis. FASEB J. 2015 Jun;29(6):2327-37

- Weiss J, Hurley LA, Harris RM, Finlayson C, Tong M, Fisher LA, Moran JL, Beier DR, Mason C, Jameson JL, ENU mutagenesis in mice identifies candidate genes for hypogonadism. Mamm Genome. 2012 Jan 19;

- Huang Z, Rivas B, Agouln k AI, Insulin-like 3 signaling is important for testicular descent but dispensable for spermatogenesis and germ cell survival in adult mice. Biol Reprod. 2012;87(6):143

- Segal MS, Sautina L, Li S, Diao Y, Agouln k AI, Kielczewski J, McGuane JT, Grant MB, Conrad KP, Relaxin increases human endothelial progenitor cell NO and migration and vasculogenesis in mice. Blood. 2012 Jan 12;119(2):629-36

- Li Z, Feng S, Lopez V, Elhammady G, Anderson ML, Kaftanovskaya EM, Agouln k AI, Uterine cysts in female mice deficient for caveolin-1 and insulin-like 3 receptor RXFP2. Endocrinology. 2011 Jun;152(6):2474-82

- Kaftanovskaya EM, Feng S, Huang Z, Tan Y, Barbara AM, Kaur S, Truong A, Gorlov IP, Agoulnik AI, Suppression of insulin-like3 receptor reveals the role of beta-catenin and Notch signaling in gubernaculum development. Mol Endocrinol. 2011 Jan;25(1):170-83

- Skarnes WC, Rosen B, West AP, Koutsourakis M, Bushell W, Iyer V, Mujica AO, Thomas M, Harrow J, Cox T, Jackson D, Severin J, Biggs P, Fu J, Nefedov M, de Jong PJ, Stewart AF, Bradley A, A conditional knockout resource for the genome-wide study of mouse gene function. Nature. 2011 Jun 16;474(7351):337- 42

- Harris RM, Finlayson C, Weiss J, Fisher L, Hurley L, Barrett T, Emge D, Bathgate RA, Agoulnik AI, Jameson JL, A missense mutation in LRR8 of RXFP2 is associated with cryptorchidism. Mamm Genome. 2010 Oct;21(9-10):442-9

- Ferlin A, Pepe A, Gianesello L, Garolla A, Feng S, Giannini S, Zaccolo M, Facciolli A, Morello R, Agoulnik AI, Foresta C, Mutations in the insulin-like factor 3 receptor are associated with osteoporosis. J Bone Miner Res. 2008 May;23(5):683-93

- Kamat AA, Feng S, Bogatcheva NV, Truong A, Bishop CE, Agoulnik AI, Genetic targeting of relaxin and insulin-like factor 3 receptors in mice. Endocrinology. 2004 Oct;145(10):4712-20

- Bogatcheva NV, Truong A, Feng S, Engel W, Adham IM, Agoulnik AI, GREAT/LGR8 Is the Only Receptor for Insulin-L ke 3 Peptide. Mol Endocrinol. 2003 Dec;17(12):2639-46

- Gorlov IP, Kamat A, Bogatcheva NV, Jones E, Lamb DJ, Truong A, Bishop CE, McElreavey K, Agouln k AI, Mutations of the GREAT gene cause cryptorchidism. Hum Mol Genet. 2002 Sep 15;11(19):2309-18

GPR107

Knockout references

http://www.mousephenotype.org/data/genes/MGI:2139054
http://www.informatics.jax.org/marker/MGI:2139054

Phenotype references

- Zhou GL, Na SY, Niedra R, Seed B, Deficits in receptor-mediated endocytosis and recycling in cells from mice with Gpr107 locus disruption. J Cell Sci. 2014 Sep 15;127(Pt 18):3916-27

- Skarnes WC, Rosen B, West AP, Koutsourakis M, Bushell W, Iyer V, Mujica AO, Thomas M, Harrow J, Cox T, Jackson D, Severin J, Biggs P, Fu J, Nefedov M, de Jong PJ, Stewart AF, Bradley A, A conditional knockout resource for the genome-wide study of mouse gene function. Nature. 2011 Jun 16;474(7351):337- 42

- Hansen GM, Markesich DC, Burnett MB, Zhu Q, Dionne KM, Richter LJ, Finnell RH, Sands AT, Zambrowicz BP, Abuin A, Large-scale gene trapping in C57BL/6N mouse embryonic stem cells. Genome Res. 2008 Oct;18(10):1670-9

- Friedel RH, Seisenberger C, Kaloff C, Wurst W, EUCOMM--the European conditional mouse mutagenesis program. Brief Funct Genomic Proteomic. 2007 Sep;6(3):180-5

- Stryke D, Kawamoto M, Huang CC, Johns SJ, King LA, Harper CA, Meng EC, Lee RE, Yee A, L'Italien L, Chuang PT, Young SG, Skarnes WC, Babbitt PC, Ferrin TE, BayGenomics: a resource of insertional mutations in mouse embryonic stem cells. Nucleic Acids Res. 2003 Jan 1;31(1):278-81

- Hansen J, Floss T, Van Sloun P, Fuchtbauer EM, Vauti F, Arnold HH, Schnutgen F, Wurst W, von Melchner H, Ruiz P, A large-scale, gene-driven mutagenesis approach for the functional analysis of the mouse genome. Proc Natl Acad Sci U S A. 2003 Aug 19;100(17):9918-22

- Zambrowicz BP, Abuin A, Ramirez-Solis R, Richter LJ, Piggott J, BeltrandelRio H, Buxton EC, Edwards J, Finch RA, Friddle CJ, Gupta A, Hansen G, Hu Y, Huang W, Jaing C, Key BW Jr, Kipp P, Kohlhauff B, Ma ZQ, Markesich D, Payne R, Potter DG, Qian N, Shaw J, Schrick J, Shi ZZ, Sparks MJ, Van Sligtenhorst I, Vogel P, Walke W, Xu N, Zhu Q, Person C, Sands AT, Wnk1 kinase deficiency lowers blood pressure in mice: a gene-trap screen to identify potential targets for therapeutic intervention. Proc Natl Acad Sci U S A. 2003 Nov 25;100(24):14109-14

GPR116

Knockout references

http://www.mousephenotype.org/data/genes/MGI:2182928
http://www.informatics.jax.org/marker/MGI:2182928

Phenotype references

- Ariestanti DM, Ando H, Hirose S, Nakamura N, Targeted Disruption of Ig-Hepta/Gpr116 Causes Emphysema-like Symptoms That Are Associated with Alveolar Macrophage Activation. J Biol Chem. 2015 Apr 24;290(17):11032-40

- Yang MY, Hilton MB, Seaman S, Haines DC, Nagashima K, Burks CM, Tessarollo L, Ivanova PT, Brown HA, Umstead TM, Floros J, Chroneos ZC, St Croix B, Essential Regulation of Lung Surfactant Homeostasis by the Orphan G Protein-Coupled Receptor GPR116. Cell Rep. 2013 May 30;3(5):1457-64

- Fukuzawa T, Ishida J, Kato A, Ichinose T, Ariestanti DM, Takahashi T, Ito K, Abe J, Suzuki T, Wakana S, Fukamizu A, Nakamura N, Hirose S, Lung surfactant levels are regulated by Ig-Hepta/GPR116 by monitoring surfactant protein D. PLoS One. 2013;8(7):e69451

- Bridges JP, Ludwig MG, Mueller M, Kinzel B, Sato A, Xu Y, Whitsett JA, Ikegami M, Orphan G protein- coupled receptor GPR116 regulates pulmonary surfactant pool size. Am J Respir Cell Mol Biol. 2013 Sep;49(3):348-57

- Skarnes WC, Rosen B, West AP, Koutsourakis M, Bushell W, Iyer V, Mujica AO, Thomas M, Harrow J, Cox T, Jackson D, Severin J, Biggs P, Fu J, Nefedov M, de Jong PJ, Stewart AF, Bradley A, A conditional knockout resource for the genome-wide study of mouse gene function. Nature. 2011 Jun 16;474(7351):337- 42

GPR120

Knockout references

http://www.mousephenotype.org/data/genes/MGI:2147577
http://www.informatics.jax.org/marker/MGI:2147577

Phenotype references

- Iwasaki K, Harada N, Sasaki K, Yamane S, Iida K, Suzuki K, Hamasaki A, Nasteska D, Shibue K, Joo E, Harada T, Hashimoto T, Asakawa Y, Hirasawa A, Inagaki N, Free fatty acid receptor GPR120 is highly expressed in enteroendocrine K cells of the upper small intestine and has a critical role in GIP secretion after fat ingestion. Endocrinology. 2015 Mar;156(3):837-46

- Ancel D, Bernard A, Subramaniam S, Hirasawa A, Tsujimoto G, Hashimoto T, Passilly-Degrace P, Khan NA, Besnard P, The oral lipid sensor GPR120 is not indispensable for the orosensory detection of dietary lipids in mice. J Lipid Res. 2015 Feb;56(2):369-78

- Stone VM, Dhayal S, Brocklehurst KJ, Lenaghan C, Sorhede Winzell M, Hammar M, Xu X, Smith DM, Morgan NG, GPR120 (FFAR4) is preferentially expressed in pancreatic delta cells and regulates somatostatin secretion from murine islets of Langerhans. Diabetologia. 2014 Jun;57(6):1182-91

- Xiong Y, Swaminath G, Cao Q, Yang L, Guo Q, Salomonis H, Lu J, Houze JB, Dransfield PJ, Wang Y, Liu JJ, Wong S, Schwandner R, Steger F, Bar bault H, Liu L, Coberly S, Miao L, Zhang J, Lin DC, Schwarz M, Activation of FFA1 mediates GLP-1 secretion in mice. Evidence for allosterism at FFA1. Mol Cell Endocrinol. 2013 Apr 30;369(1-2):119-29

- Godinot N, Yasumatsu K, Barcos ME, Pineau N, Ledda M, Viton F, Ninomiya Y, le Coutre J, Damak S, Activation of tongue-expressed GPR40 and GPR120 by non caloric agonists is not sufficient to drive preference in mice. Neuroscience. 2013 Oct 10;250:20-30

- Sclafani A, Zukerman S, Ackroff K, GPR40 and GPR120 fatty acid sensors are critical for postoral but not oral mediation of fat preferences in the mouse. Am J Physiol Regul Integr Comp Physiol. 2013 Dec 15;305(12):R1490-7

- Ichimura A, Hirasawa A, Poulain-Godefroy O, Bonnefond A, Hara T, Yengo L, Kimura I, Leloire A, Liu N, Iida K, Choquet H, Besnard P, Lecoeur C, Vivequin S, Ayukawa K, Takeuchi M, Ozawa K, Tauber M, Maffeis C, Morandi A, Buzzetti R, Elliott P, Pouta A, Jarvelin MR, Korner A, Kiess W, Pigeyre M, Caiazzo R, Van Hul W, Van Gaal L, Horber F, Balkau B, Levy-Marchal C, Rouskas K, Kouvatsi A, Hebebrand J, Hinney A, Scherag A, Pattou F, Meyre D, Koshimizu TA, Wolowczuk I, Tsujimoto G, Froguel P, Dysfunction of lipid sensor GPR120 leads to obesity in both mouse and human. Nature. 2012 Mar 15;483(7389):350-4

- Skarnes WC, Rosen B, West AP, Koutsourakis M, Bushell W, Iyer V, Mujica AO, Thomas M, Harrow J, Cox T, Jackson D, Severin J, Biggs P, Fu J, Nefedov M, de Jong PJ, Stewart AF, Bradley A, A conditional knockout resource for the genome-wide study of mouse gene function. Nature. 2011 Jun 16;474(7351):337- 42

- Cartoni C, Yasumatsu K, Ohkuri T, Shigemura N, Yoshida R, Godinot N, le Coutre J, Ninomiya Y, Damak S, Taste preference for fatty acids is mediated by GPR40 and GPR120. J Neurosci. 2010 Jun 23;30(25):8376- 82

- Zambrowicz BP, Abuin A, Ramirez-Solis R, Richter LJ, Piggott J, BeltrandelRio H, Buxton EC, Edwards J, Finch RA, Friddle CJ, Gupta A, Hansen G, Hu Y, Huang W, Jaing C, Key BW Jr, Kipp P, Kohlhauff B, Ma ZQ, Markesich D, Payne R, Potter DG, Qian N, Shaw J, Schrick J, Shi ZZ, Sparks MJ, Van Sligtenhorst I, Vogel P, Walke W, Xu N, Zhu Q, Person C, Sands AT, Wnk1 kinase deficiency lowers blood pressure in mice: a gene-trap screen to identify potential targets for therapeutic intervention. Proc Natl Acad Sci U S A. 2003 Nov 25;100(24):14109-14

GPR124

Knockout references

http://www.mousephenotype.org/data/genes/MGI:1925810
http://www.informatics.jax.org/marker/MGI:1925810

Phenotype references

- Posokhova E, Shukla A, Seaman S, Volate S, Hilton MB, Wu B, Morris H, Swing DA, Zhou M, Zudaire E, Rubin JS, St Croix B, GPR124 functions as a WNT7-specific coactivator of canonical beta-catenin signaling. Cell Rep. 2015 Jan 13;10(2):123-30

- Economides AN, Frendewey D, Yang P, Dominguez MG, Dore AT, Lobov IB, Persaud T, Rojas J, McClain J, Lengyel P, Droguett G, Chernomorsky R, Stevens S, Auerbach W, Dechiara TM, Pouyemirou W, Cruz JM Jr, Feeley K, Mellis IA, Yasenchack J, Hatsell SJ, Xie L,Latres E, Huang L, Zhang Y, Pefanis E, Skokos D, Deckelbaum RA, Croll SD, Davis S, Valenzuela DM, Gale NW, Murphy AJ, Yancopoulos GD, Conditionals by inversion provide a universal method for the generation of conditional alleles. Proc Natl Acad Sci U S A. 2013 Aug 20;110(34):E3179-88

- Anderson KD, Pan L, Yang XM, Hughes VC, Walls JR, Dominguez MG, Simmons MV, Burfeind P, Xue Y, Wei Y, Macdonald LE, Thurston G, Daly C, Lin HC, Economides AN, Valenzuela DM, Murphy AJ, Yancopoulos GD, Gale NW, Angiogenic sprouting into neural tissue requires Gpr124, an orphan G protein- coupled receptor. Proc Natl Acad Sci U S A. 2011 Feb 15;108(7):2807-12

- Cullen M, Elzarrad MK, Seaman S, Zudaire E, Stevens J, Yang MY, Li X, Chaudhary A, Xu L, Hilton MB, Logsdon D, Hsiao E, Stein EV, Cuttitta F, Haines DC, Nagashima K, Tessarollo L, St Croix B, GPR124, an

orphan G protein-coupled receptor, is required for CNS-specific vascularization and establishment of the blood-brain barrier. Proc Natl Acad Sci U S A. 2011 Apr 5;108(14):5759-64

- Kuhnert F, Mancuso MR, Shamloo A, Wang HT, Choksi V, Florek M, Su H, Fruttiger M, Young WL, Heilshorn SC, Kuo CJ, Essential regulation of CNS angiogenesis by the orphan G protein-coupled receptor GPR124. Science. 2010 Nov 12;330(6006):985-9

- Hansen GM, Markesich DC, Burnett MB, Zhu Q, Dionne KM, Richter LJ, Finnell RH, Sands AT, Zambrowicz BP, Abuin A, Large-scale gene trapping in C57BL/6N mouse embryonic stem cells. Genome Res. 2008 Oct;18(10):1670-9

- Mouse Genome Informatics (MGI) and National Center for Biotechnology Information (NCBI), Mouse Gene Trap Data Load from dbGSS. Database Download. 2008;

- Zambrowicz BP, Abuin A, Ramirez-Solis R, Richter LJ, Piggott J, BeltrandelRio H, Buxton EC, Edwards J, Finch RA, Friddle CJ, Gupta A, Hansen G, Hu Y, Huang W, Jaing C, Key BW Jr, Kipp P, Kohlhauff B, Ma ZQ, Markesich D, Payne R, Potter DG, Qian N, Shaw J, Schrick J, Shi ZZ, Sparks MJ, Van Sligtenhorst I, Vogel P, Walke W, Xu N, Zhu Q, Person C, Sands AT, Wnk1 kinase deficiency lowers blood pressure in mice: a gene-trap screen to identify potential targets for therapeutic intervention. Proc Natl Acad Sci U S A. 2003 Nov 25;100(24):14109-14

- Valenzuela DM, Murphy AJ, Frendewey D, Gale NW, Economides AN, Auerbach W, Poueymirou WT, Adams NC, Rojas J, Yasenchak J, Chernomorsky R, Boucher M, Elsasser AL, Esau L, Zheng J, Griffiths JA, Wang X, Su H, Xue Y, Dominguez MG, Noguera I, Torres R, Macdonald LE, Stewart AF, DeChiara TM, Yancopoulos GD, High-throughput engineering of the mouse genome coupled with high-resolution expression analysis. Nat Biotechnol. 2003 Jun;21(6):652-9

GPR126

Knockout references

http://www.mousephenotype.org/data/genes/MGI:1916151
http://www.informatics.jax.org/marker/MGI:1916151

Phenotype references

- Karner CM, Long F, Solnica-Krezel L, Monk KR, Gray RS, Gpr126/Adgrg6 deletion in cartilage models idiopathic scoliosis and pectus excavatum in mice. Hum Mol Genet. 2015 Aug 1;24(15):4365-73

- Mogha A, Benesh AE, Patra C, Engel FB, Schoneberg T, Liebscher I, Monk KR, Gpr126 functions in Schwann cells to control differentiation and myelination via G-protein activation. J Neurosci. 2013 Nov 13;33(46):17976-85

- Patra C, van Amerongen MJ, Ghosh S, Ricciardi F, Sajjad A, Novoyatleva T, Mogha A, Monk KR, Muhlfeld C, Engel FB, Organ-specific function of adhesion G protein-coupled receptor GPR126 is domain-dependent. Proc Natl Acad Sci U S A. 2013 Oct 15;110(42):16898-903

- Monk KR, Oshima K, Jors S, Heller S, Talbot WS, Gpr126 is essential for peripheral nerve development and myelination in mammals. Development. 2011 Jul;138(13):2673-80

- Skarnes WC, Rosen B, West AP, Koutsourakis M, Bushell W, Iyer V, Mujica AO, Thomas M, Harrow J, Cox T, Jackson D, Severin J, Biggs P, Fu J, Nefedov M, de Jong PJ, Stewart AF, Bradley A, A conditional knockout resource for the genome-wide study of mouse gene function. Nature. 2011 Jun 16;474(7351):337- 42

- Waller-Evans H, Promel S, Langenhan T, Dixon J, Zahn D, Colledge WH, Doran J, Carlton MB, Davies B, Aparicio SA, Grosse J, Russ AP, The orphan adhesion-GPCR GPR126 is required for embryonic development in the mouse. PLoS One. 2010;5(11):e14047

GPR131

Knockout references

http://www.mousephenotype.org/data/genes/MGI:2653863
http://www.informatics.jax.org/marker/MGI:2653863

Phenotype references

- Renga B, Bucci M, Cipriani S, Carino A, Monti MC, Zampella A, Gargiulo A, d'Emmanuele di Villa Bianca R, Distrutti E, Fiorucci S, Cystathionine gamma-lyase, a H2S-generating enzyme, is a GPBAR1-regulated gene and contr butes to vasodilation caused by secondary bile acids. Am J Physiol Heart Circ Physiol. 2015 Jul 1;309(1):H114-26

- Lou G, Ma X, Fu X, Meng Z, Zhang W, Wang YD, Van Ness C, Yu D, Xu R, Huang W, GPBAR1/TGR5 mediates bile acid-induced cytokine expression in murine Kupffer cells. PLoS One. 2014;9(4):e93567

- Perino A, Pols TW, Nomura M, Stein S, Pellicciari R, Schoonjans K, TGR5 reduces macrophage migration through mTOR-induced C/EBPbeta differential translation. J Clin Invest. 2014 Dec;124(12):5424-36

- Rajagopal S, Kumar DP, Mahavadi S, Bhattacharya S, Zhou R, Corvera CU, Bunnett NW, Grider JR, Murthy KS, Activation of G protein-coupled bile acid receptor, TGR5, induces smooth muscle relaxation via both Epac- and PKA-mediated inh bition of RhoA/Rho kinase pathway. Am J Physiol Gastrointest Liver Physiol. 2013 Mar 1;304(5):G527-35

- Alemi F, Kwon E, Poole DP, Lieu T, Lyo V, Cattaruzza F, Cevikbas F, Steinhoff M, Nassini R, Materazzi S, Guerrero-Alba R, Valdez-Morales E, Cottrell GS, Schoonjans K, Geppetti P, Vanner SJ, Bunnett NW, Corvera CU, The TGR5 receptor mediates bile acid-induced itch and analgesia. J Clin Invest. 2013 Apr 1;123(4):1513-30

- Harach T, Pols TW, Nomura M, Maida A, Watanabe M, Auwerx J, Schoonjans K, TGR5 potentiates GLP-1 secretion in response to anionic exchange resins. Sci Rep. 2012;2:430

- Pols TW, Nomura M, Harach T, Lo Sasso G, Oosterveer MH, Thomas C, Rizzo G, Gioiello A, Adorini L, Pellicciari R, Auwerx J, Schoonjans K, TGR5 activation inhibits atherosclerosis by reducing macrophage inflammation and lipid loading. Cell Metab. 2011 Dec 7;14(6):747-57

- Cipriani S, Mencarelli A, Chini MG, Distrutti E, Renga B, Bifulco G, Baldelli F, Donini A, Fiorucci S, The Bile Acid Receptor GPBAR-1 (TGR5) Modulates Integrity of Intestinal Barrier and Immune Response to Experimental Colitis. PLoS One. 2011;6(10):e25637

- Skarnes WC, Rosen B, West AP, Koutsourakis M, Bushell W, Iyer V, Mujica AO, Thomas M, Harrow J, Cox T, Jackson D, Severin J, Biggs P, Fu J, Nefedov M, de Jong PJ, Stewart AF, Bradley A, A conditional knockout resource for the genome-wide study of mouse gene function. Nature. 2011 Jun 16;474(7351):337- 42

- Lavoie B, Balemba OB, Godfrey C, Watson CA, Vassileva G, Corvera CU, Nelson MT, Mawe GM, Hydrophobic bile salts inhibit gal bladder smooth muscle function via stimulation of GPBAR1 receptors and activation of KATP channels. J Physiol. 2010 Sep 1;588(Pt 17):3295-305

GPR132

Knockout references

http://www.mousephenotype.org/data/genes/MGI:1890220
http://www.informatics.jax.org/marker/MGI:1890220

Phenotype references

Frasch SC, Fernandez-Boyanapalli RF, Berry KA, Murphy RC, Leslie CC, Nick JA, Henson PM, Bratton DL, Neutrophils regulate tissue Neutrophilia in inflammation via the oxidant-modified lipid lysophosphatidylserine. J Biol Chem. 2013 Feb 15;288(7):4583-93

Parks BW, Black LL, Zimmerman KA, Metz AE, Steele C, Murphy-Ullrich JE, Kabarowski JH, CD36, but not G2A, modulates efferocytosis, inflammation, and fibrosis following bleomycin-induced lung injury. J Lipid Res. 2013 Apr;54(4):1114-23

Palm NW, Rosenstein RK, Yu S, Schenten DD, Florsheim E, Medzhitov R, Bee venom phospholipase A2 induces a primary type 2 response that is dependent on the receptor ST2 and confers protective immunity. Immunity. 2013 Nov 14;39(5):976-85

Skarnes WC, Rosen B, West AP, Koutsourakis M, Bushell W, Iyer V, Mujica AO, Thomas M, Harrow J, Cox T, Jackson D, Severin J, Biggs P, Fu J, Nefedov M, de Jong PJ, Stewart AF, Bradley A, A conditional knockout resource for the genome-wide study of mouse gene function. Nature. 2011 Jun 16;474(7351):337-42

Parks BW, Srivastava R, Yu S, Kabarowski JH, ApoE-dependent modulation of HDL and atherosclerosis by G2A in LDL receptor-deficient mice independent of bone marrow-derived cells. Arterioscler Thromb Vasc Biol. 2009 Apr;29(4):539-47

Bolick DT, Skaflen MD, Johnson LE, Kwon SC, Howatt D, Daugherty A, Ravichandran KS, Hedrick CC, G2A deficiency in mice promotes macrophage activation and atherosclerosis. Circ Res. 2009 Feb 13;104(3):318-27

Bolick DT, Whetzel AM, Skaflen M, Deem TL, Lee J, Hedrick CC, Absence of the G protein-coupled receptor G2A in mice promotes monocyte/endothelial interactions in aorta. Circ Res. 2007 Mar 2;100(4):572-80

Parks BW, Lusis AJ, Kabarowski JH, Loss of the lysophosphatidylcholine effector, G2A, ameliorates aortic atherosclerosis in low-density lipoprotein receptor knockout mice. Arterioscler Thromb Vasc Biol. 2006 Dec;26(12):2703-9

Radu CG, Nijagal A, McLaughlin J, Wang L, Witte ON, Differential proton sensitivity of related G protein-coupled receptors T cell death-associated gene 8 and G2A expressed in immune cells. Proc Natl Acad Sci U S A. 2005 Feb 1;102(5):1632-7

Lang LV, Radu CG, Wang L, Riedinger M, Witte ON, Gi-independent macrophage chemotaxis to lysophosphatidylcholine via the immunoregulatory GPCR G2A. Blood. 2005 Feb 1;105(3):1127-34

Parks BW, Gambill GP, Lusis AJ, Kabarowski JH, Loss of G2A promotes macrophage accumulation in atherosclerotic lesions of low density lipoprotein receptor-deficient mice. J Lipid Res. 2005 Jul;46(7):1405-15

Le LQ, Kabarowski JH, Weng Z, Satterthwaite AB, Harvill ET, Jensen ER, Miller JF, Witte ON, Mice lacking the orphan G protein-coupled receptor G2A develop a late-onset autoimmune syndrome. Immunity. 2001 May;14(5):561-71

GPR143

Knockout references

http://www.mousephenotype.org/data/genes/MGI:107193
http://www.informatics.jax.org/marker/MGI:107193

Phenotype references

- Rachel RA, Nagashima K, O'Sullivan TN, Frost LS, Stefano FP, Marigo V, Boesze-Battaglia K, Melanoregulin, product of the dsu locus, links the BLOC-pathway and OA1 in organelle biogenesis. PLoS One. 2012;7(9):e42446

- Young A, Jiang M, Wang Y, Ahmedli NB, Ramirez J, Reese BE, Birnbaumer L, Farber DB, Specific interaction of Galphai3 with the Oa1 G-protein coupled receptor controls the size and density of melanosomes in retinal pigment epithelium. PLoS One. 2011;6(9):e24376

- Skarnes WC, Rosen B, West AP, Koutsourakis M, Bushell W, Iyer V, Mujica AO, Thomas M, Harrow J, Cox T, Jackson D, Severin J, Biggs P, Fu J, Nefedov M, de Jong PJ, Stewart AF, Bradley A, A conditional knockout resource for the genome-wide study of mouse gene function. Nature. 2011 Jun 16;474(7351):337- 42

- Young A, Powelson EB, Whitney IE, Raven MA, Nusinowitz S, Jiang M, Birnbaumer L, Reese BE, Farber DB, Involvement of OA1, an intracellular GPCR, and G alpha i3, its binding protein, in melanosomal biogenesis and optic pathway formation. Invest Ophthalmol Vis Sci. 2008 Jul;49(7):3245-52

- Palmisano I, Bagnato P, Palmigiano A, Innamorati G, Rotondo G, Altimare D, Venturi C, Sviderskaya EV, Piccirillo R, Coppola M, Marigo V, Incerti B, Ballabio A, Surace EM, Tacchetti C, Bennett DC, Schiaffino MV, The ocular albinism type 1 protein, an intracellular G protein-coupled receptor, regulates melanosome transport in pigment cells. Hum Mol Genet. 2008 Nov 15;17(22):3487-501

- Cortese K, Giordano F, Surace EM, Venturi C, Ballabio A, Tacchetti C, Marigo V, The ocular a binism type 1 (OA1) gene controls melanosome maturation and size. Invest Ophthalmol Vis Sci. 2005 Dec;46(12):4358-64

- Incerti B, Cortese K, Pizzigoni A, Surace EM, Varani S, Coppola M, Jeffery G, Seeliger M, Jaissle G, Bennett DC, Marigo V, Schiaffino MV, Tacchetti C, Ballabio A, Oa1 knock-out: new insights on the pathogenesis of ocular a binism type 1. Hum Mol Genet. 2000 Nov 22;9(19):2781-8

GPR149

Knockout references

http://www.mousephenotype.org/data/genes/MGI:2443628;
http://www.informatics.jax.org/allele/MGI:5473295?page=alleleDetail&id=MGI:5473295

Phenotype references

- Skarnes WC, Rosen B, West AP, Koutsourakis M, Bushell W, Iyer V, Mujica AO, Thomas M, Harrow J, Cox T, Jackson D, Severin J, Biggs P, Fu J, Nefedov M, de Jong PJ, Stewart AF, Bradley A, A conditional knockout resource for the genome-wide study of mouse gene function. Nature. 2011 Jun 16;474(7351):337- 42

- Edson MA, Lin YN, Matzuk MM, Deletion of the novel oocyte-enriched gene, Gpr149, leads to increased fertility in mice. Endocrinology. 2010 Jan;151(1):358-68

- Zambrowicz BP, Abuin A, Ramirez-Solis R, Richter LJ, Piggott J, BeltrandelRio H, Buxton EC, Edwards J, Finch RA, Friddle CJ, Gupta A, Hansen G, Hu Y, Huang W, Jaing C, Key BW Jr, Kipp P, Kohlhauff B, Ma ZQ, Markesich D, Payne R, Potter DG, Qian N, Shaw J, Schrick J, Shi ZZ, Sparks MJ, Van Sligtenhorst I, Vogel P, Walke W, Xu N, Zhu Q, Person C, Sands AT, Wnk1 kinase deficiency lowers blood pressure in mice: a gene-trap screen to identify potential targets for therapeutic intervention. Proc Natl Acad Sci U S A. 2003 Nov 25;100(24):14109-14

- Cook SA, Akeson EC, Calvano C, Johnson KR, Mandell J, Hawes NL, Bronson RT, Roderick TH, Davisson MT, Mouse paracentric inversion In(3)55Rk mutates the urate oxidase gene. Cytogenet Cell Genet. 2001;93(1-2):77-82

- Lyon MF, Rastan S, Brown SDM, in Lyon MF, Rastan S, Brown SDM (eds), Genetic Variants and Strains of the Laboratory Mouse. 3rd Edition. 1996Oxford: Oxford University Press.

- Roderick TH, Using inversions to detect and study recessive lethals and detrimentals in mice, in de Serres FJ, Sheridan W (eds), Utilization of Mammalian Specific Locus Studies in Hazard Evaluation and Estimation of Genetic Risk. 1983:135-67. Plenum Publishing Corp.

GPR154

Knockout references

http://www.mousephenotype.org/data/genes/MGI:2441738
http://www.informatics.jax.org/marker/MGI:2441738

Phenotype references

- Ruzza C, Pulga A, Rizzi A, Marzola G, Guerrini R, Calo' G, Behavioural phenotypic characterization of CD-1 mice lacking the neuropeptide S receptor. Neuropharmacology. 2012 Apr;62(5-6):1999-2009

- Pulga A, Ruzza C, Rizzi A, Guerrini R, Calo G, Anxiolytic- and panicolytic-like effects of Neuropeptide S in the mouse elevated T-maze. Eur J Neurosci. 2012 Dec;36(11):3531-7

- Skarnes WC, Rosen B, West AP, Koutsourakis M, Bushell W, Iyer V, Mujica AO, Thomas M, Harrow J, Cox T, Jackson D, Severin J, Biggs P, Fu J, Nefedov M, de Jong PJ, Stewart AF, Bradley A, A conditional knockout resource for the genome-wide study of mouse gene function. Nature. 2011 Jun 16;474(7351):337- 42

- Duangdao DM, Clark SD, Okamura N, Reinscheid RK, Behavioral phenotyping of neuropeptide S receptor knockout mice. Behav Brain Res. 2009 Dec 14;205(1):1-9

- Allen IC, Pace AJ, Jania LA, Ledford JG, Latour AM, Snouwaert JN, Bernier V, Stocco R, Therien AG, Koller BH, Expression and function of NPSR1/GPRA in the lung before and after induction of asthma-like disease. Am J Physiol Lung Cell Mol Physiol. 2006 Nov;291(5):L1005-17

- Zambrowicz BP, Abuin A, Ramirez-Solis R, Richter LJ, Piggott J, BeltrandelRio H, Buxton EC, Edwards J, Finch RA, Friddle CJ, Gupta A, Hansen G, Hu Y, Huang W, Jaing C, Key BW Jr, Kipp P, Kohlhauff B, Ma ZQ, Markesich D, Payne R, Potter DG, Qian N, Shaw J, Schrick J, Shi ZZ, Sparks MJ, Van Sligtenhorst I, Vogel P, Walke W, Xu N, Zhu Q, Person C, Sands AT, Wnk1 kinase deficiency lowers blood pressure in mice: a gene-trap screen to identify potential targets for therapeutic intervention. Proc Natl Acad Sci U S A. 2003 Nov 25;100(24):14109-14

GPR161

Knockout references

http://www.mousephenotype.org/data/genes/MGI:2685054
http://www.informatics.jax.org/marker/MGI:2685054

Phenotype references

- Li BI, Matteson PG, Ababon MF, Nato AQ Jr, Lin Y, Nanda V, Matise TC, Millonig JH, The orphan GPCR, Gpr161, regulates the retinoic acid and canonical Wnt pathways during neurulation. Dev Biol. 2015 Jun 1;402(1):17-31

- Mukhopadhyay S, Wen X, Ratti N, Loktev A, Rangell L, Scales SJ, Jackson PK, The ciliary G-protein-coupled receptor Gpr161 negatively regulates the Sonic hedgehog pathway via cAMP signaling. Cell. 2013 Jan 17;152(1-2):210-23

- Matteson PG, Desai J, Korstanje R, Lazar G, Borsuk TE, Rollins J, Kadambi S, Joseph J, Rahman T, Wink J, Benayed R, Paigen B, Millonig JH, The orphan G protein-coupled receptor, Gpr161, encodes the vacuolated lens locus and controls neurulation and lens development. Proc Natl Acad Sci U S A. 2008 Feb 12;105(6):2088-93

- Lyon MF, Rastan S, Brown SDM, in Lyon MF, Rastan S, Brown SDM (eds), Genetic Variants and Strains of the Laboratory Mouse. 3rd Edition. 1996Oxford: Oxford University Press.

- Wilson DB, Wyatt DP, In vitro expression of neural tube pathology in the vl mutant mouse. J Neuropathol Exp Neurol. 1993 May;52(3):253-9

- Wilson DB, Wyatt DP, Aberrant convergence of the neural folds in the mouse mutant vl. Teratology. 1992 Jan;45(1):105-12

- Swank RT, Reddington M, Howlett O, Novak EK, Platelet storage pool deficiency associated with inherited abnormalities of the inner ear in the mouse pigment mutants muted and mocha. Blood. 1991 Oct 15;78(8):2036-44

- Wilson DB, Wyatt DP, Ultrastructural defects in the apical neural folds in mutant embryos with spina bifida. Acta Neuropathol. 1989;79(1):94-100

- Wilson DB, Wyatt DP, Closure of the posterior neuropore in the vl mutant mouse. Anat Embryol (Berl). 1988;178(6):559-63

- Roderick TH, Using inversions to detect and study recessive lethals and detrimentals in mice, in de Serres FJ, Sheridan W (eds), Utilization of Mammalian Specific Locus Studies in Hazard Evaluation and Estimation of Genetic Risk. 1983:135-67. Plenum Publishing Corp..

- Dickie MM, Vacuolated lens. Mouse News Lett. 1967;36:39-40

GPR179

Knockout references

http://www.mousephenotype.org/data/genes/MGI:2443409

http://www.informatics.jax.org/marker/MGI:2443409

Phenotype references

- Chang B, Survey of the mutation in C3H substrains. Mol Vis. 2015;21:1101-1105
- Ray TA, Heath KM, Hasan N, Noel JM, Samuels IS, Martemyanov KA, Peachey NS, McCall MA, Gregg RG, GPR179 is required for high sensitivity of the mGluR6 signaling cascade in depolarizing bipolar cells. J Neurosci. 2014 Apr 30;34(18):6334-43
- Klooster J, van Genderen MM, Yu M, Florijn RJ, Riemslag FC, Bergen AA, Gregg RG, Peachey NS, Kamermans M, Ultrastructural localization of GPR179 and the impact of mutant forms on retinal function in CSNB1 patients and a mouse model. Invest Ophthalmol Vis Sci. 2013;54(10):6973-81
- Peachey NS, Ray TA, Florijn R, Rowe LB, Sjoerdsma T, Contreras-Alcantara S, Baba K, Tosini G, Pozdeyev N, Iuvone PM, Bojang P Jr, Pearring JN, Simonsz HJ, van Genderen M, Birch DG, Traboulsi EI, Dorfman A, Lopez I, Ren H, Goldberg AF, Nishina PM, Lachapelle P, McCall MA, Koenekoop RK, Bergen AA, Kamermans M, Gregg RG, GPR179 is required for depolarizing bipolar cell function and is mutated in autosomal-recessive complete congenital stationary night blindness. Am J Hum Genet. 2012 Feb 10;90(2):331-9
- Orlandi C, Posokhova E, Masuho I, Ray TA, Hasan N, Gregg RG, Martemyanov KA, GPR158/179 regulate G protein signaling by controlling localization and activity of the RGS7 complexes. J Cell Biol. 2012 Jun 11;197(6):711-9
- Peachey NS, Pearring JN, Bojang P Jr, Hirschtritt ME, Sturgill-Short G, Ray TA, Furukawa T, Koike C, Goldberg AF, Shen Y, McCall MA, Nawy S, Nishina PM, Gregg RG, Depolarizing bipolar cell dysfunction due to a Trpm1 point mutation. J Neurophysiol. 2012 Nov;108(9):2442-51
- Terzian T, Dumble M, Arbab F, Thaller C, Donehower LA, Lozano G, Justice MJ, Roop DR, Box NF, Rpl27a mutation in the sooty foot ataxia mouse phenocopies high p53 mouse models. J Pathol. 2011 Aug;224(4):540-52
- Boles MK, Wi kinson BM, Wilming LG, Liu B, Probst FJ, Harrow J, Grafham D, Hentges KE, Woodward LP, Maxwell A, Mitchell K, Risley MD, Johnson R, Hirschi K, Lupski JR, Funato Y, M ki H, Marin-Garcia P, Matthews L, Coffey AJ, Parker A, Hubbard TJ, Rogers J,Bradley A, Adams DJ, Justice MJ, Discovery of candidate disease genes in ENU-induced mouse mutants by large-scale sequencing, including a splice-site mutation in nucleoredoxin. PLoS Genet. 2009 Dec;5(12):e1000759
- Clark AT, Firozi K, Justice MJ, Mutations in a novel locus on mouse chromosome 11 resulting in male infertility associated with defects in microtubule assembly and sperm tail function. Biol Reprod. 2004 May;70(5):1317-24
- Kile BT, Hentges KE, Clark AT, Nakamura H, Salinger AP, Liu B, Box N, Stockton DW, Johnson RL, Behringer RR, Bradley A, Justice MJ, Functional genetic analysis of mouse chromosome 11. Nature. 2003 Sep 4;425(6953):81-6
- Zambrowicz BP, Abuin A, Ramirez-Solis R, Richter LJ, Piggott J, BeltrandelRio H, Buxton EC, Edwards J, Finch RA, Friddle CJ, Gupta A, Hansen G, Hu Y, Huang W, Jaing C, Key BW Jr, Kipp P, Kohlhauff B, Ma ZQ, Markesich D, Payne R, Potter DG, Qian N, Shaw J, Schrick J, Shi ZZ, Sparks MJ, Van Sligtenhorst I, Vogel P, Walke W, Xu N, Zhu Q, Person C, Sands AT, Wnk1 kinase deficiency lowers blood pressure in mice: a gene-trap screen to identify potential targets for therapeutic intervention. Proc Natl Acad Sci U S A. 2003 Nov 25;100(24):14109-14
- Zheng B, Sage M, Cai WW, Thompson DM, Tavsanli BC, Cheah YC, Bradley A, Engineering a mouse balancer chromosome. Nat Genet. 1999 Aug;22(4):375-8

GPR180

Knockout references

http://www.mousephenotype.org/data/genes/MGI:1930949
http://www.informatics.jax.org/marker/MGI:1930949

Phenotype references

- Skarnes WC, Rosen B, West AP, Koutsourakis M, Bushell W, Iyer V, Mujica AO, Thomas M, Harrow J, Cox T, Jackson D, Severin J, Biggs P, Fu J, Nefedov M, de Jong PJ, Stewart AF, Bradley A, A conditional knockout resource for the genome-wide study of mouse gene function. Nature. 2011 Jun 16;474(7351):337- 42

- Tsukada S, Iwai M, Nishiu J, Itoh M, Tomoike H, Horiuchi M, Nakamura Y, Tanaka T, Inhibition of experimental intimal thickening in mice lacking a novel G-protein-coupled receptor. Circulation. 2003 Jan 21;107(2):313-9

- Roix J, Hagge-Greenberg A, Bissonnette D, Rodick S, Russell L, O'Brien T, Molecular and functional mapping of the piebald deletion complex on mouse chromosome 14. Genetics. 2001 Feb;157(2):803-15

- Lyon MF, Rastan S, Brown SDM, in Lyon MF, Rastan S, Brown SDM (eds), Genetic Variants and Strains of the Laboratory Mouse. 3rd Edition. 1996Oxford: Oxford University Press.

- Roderick TH, Using inversions to detect and study recessive lethals and detrimentals in mice, in de Serres FJ, Sheridan W (eds), Utilization of Mammalian Specific Locus Studies in Hazard Evaluation and Estimation of Genetic Risk. 1983:135-67. Plenum Publishing Corp..

GPR183

Knockout references

http://www.mousephenotype.org/data/genes/MGI:2442034
http://www.informatics.jax.org/marker/MGI:2442034

Phenotype references

- Heinig K, Gatjen M, Grau M, Stache V, Anagnostopoulos I, Gerlach K, Niesner RA, Cseresnyes Z, Hauser AE, Lenz P, Hehlgans T, Brink R, Westermann J, Dorken B, Lipp M, Lenz G, Rehm A, Hopken UE, Access to follicular dendritic cells is a pivotal step in murine chronic lymphocytic leukemia B-cell activation and proliferation. Cancer Discov. 2014 Dec;4(12):1448-65

- Gatto D, Wood K, Caminschi I, Murphy-Durland D, Schofield P, Christ D, Karupiah G, Brink R, The chemotactic receptor EBI2 regulates the homeostasis, localization and immunological function of splenic dendritic cells. Nat Immunol. 2013 May;14(5):446-53

- Yi T, Cyster JG, EBI2-mediated bridging channel positioning supports splenic dendritic cell homeostasis and particulate antigen capture. Elife. 2013;2:e00757

- Chiang EY, Johnston RJ, Grogan JL, EBI2 is a negative regulator of type I interferons in plasmacytoid and myeloid dendritic cells. PLoS One. 2013;8(12):e83457

- Yi T, Wang X, Kelly LM, An J, Xu Y, Sailer AW, Gustafsson JA, Russell DW, Cyster JG, Oxysterol Gradient Generation by Lymphoid Stromal Cells Guides Activated B Cell Movement during Humoral Responses. Immunity. 2012 Sep 21;37(3):535-48

- Wang X, Cho B, Suzuki K, Xu Y, Green JA, An J, Cyster JG, Follicular dendritic cells help establish follicle identity and promote B cell retention in germinal centers. J Exp Med. 2011 Nov 21;208(12):2497-510

- Kelly LM, Pereira JP, Yi T, Xu Y, Cyster JG, EBI2 guides serial movements of activated B cells and ligand activity is detectable in lymphoid and nonlymphoid tissues. J Immunol. 2011 Sep 15;187(6):3026-32

- Pereira JP, Kelly LM, Xu Y, Cyster JG, EBI2 mediates B cell segregation between the outer and centre follicle. Nature. 2009 Jul 13;

- Gatto D, Paus D, Basten A, Mackay CR, Brink R, Guidance of B cells by the orphan G protein-coupled receptor EBI2 shapes humoral immune responses. Immunity. 2009 Aug 21;31(2):259-69

GRM1

Knockout references

http://www.mousephenotype.org/data/genes/MGI:1351338
http://www.informatics.jax.org/marker/MGI:1351338

Phenotype references

- Maltecca F, Baseggio E, Consolato F, Mazza D, Podini P, Young SM Jr, Drago I, Bahr BA, Puliti A, Codazzi F, Quattrini A, Casari G, Purkinje neuron Ca2+ influx reduction rescues ataxia in SCA28 model. J Clin Invest. 2015 Jan;125(1):263-74

- Reyna C, Russell J, SoRelle J, Murray AR, Beutler B, Mutagenetix entry for donald. MGI Direct Data Submission. 2015;

- Mairhofer DG, Ortner D, Tripp CH, Schaffenrath S, Fleming V, Heger L, Komenda K, Reider D, Dudziak D, Chen S, Becker JC, Flacher V, Stoitzner P, Impaired gp100-Specific CD8(+) T-Cell Responses in the Presence of Myeloid-Derived Suppressor Cells in a Spontaneous Mouse Melanoma Model. J Invest Dermatol. 2015 Nov;135(11):2785-93

- Milanese M, Giribaldi F, Melone M, Bonifacino T, Musante I, Carminati E, Rossi PI, Vergani L, Voci A, Conti F, Puliti A, Bonanno G, Knocking down metabotropic glutamate receptor 1 improves survival and disease progression in the SOD1(G93A) mouse model of amyotrophic lateral sclerosis. Neurobiol Dis. 2014 Apr;64:48-59

- Ohtani Y, Miyata M, Hashimoto K, Tabata T, Kishimoto Y, Fukaya M, Kase D, Kassai H, Nakao K, Hirata T, Watanabe M, Kano M, Aiba A, The synaptic targeting of mGluR1 by its carboxyl-terminal domain is crucial for cerebellar function. J Neurosci. 2014 Feb 12;34(7):2702-12

- Park JM, Hu JH, Milshteyn A, Zhang PW, Moore CG, Park S, Datko MC, Domingo RD, Reyes CM, Wang XJ, Etzkorn FA, Xiao B, Szumlinski KK, Kern D, Linden DJ, Worley PF, A prolyl-isomerase mediates dopamine-dependent plasticity and cocaine motor sensitization. Cell. 2013 Aug 1;154(3):637-50

- Cronin JC, Watkins-Chow DE, Incao A, Hasskamp JH, Schonewolf N, Aoude LG, Hayward NK, Bastian BC, Dummer R, Loftus SK, Pavan WJ, SOX10 ablation arrests cell cycle, induces senescence, and suppresses melanomagenesis. Cancer Res. 2013 Sep 15;73(18):5709-18

- Rossi PI, Musante I, Summa M, Pittaluga A, Emionite L, Ikehata M, Rastaldi MP, Ravazzolo R, Puliti A, Compensatory molecular and functional mechanisms in nervous system of the Grm1(crv4) mouse lacking the mGlu1 receptor: a model for motor coordination deficits. Cereb Cortex. 2013 Sep;23(9):2179-89

- Kato AS, Knierman MD, Siuda ER, Isaac JT, Nisenbaum ES, Bredt DS, Glutamate receptor delta2 associates with metabotropic glutamate receptor 1 (mGluR1), protein kinase Cgamma, and canonical transient receptor potential 3 and regulates mGluR1-mediated synaptic transmission in cerebellar Purkinje neurons. J Neurosci. 2012 Oct 31;32(44):15296-308

- Hirata T, Kumada T, Kawasaki T, Furukawa T, A ba A, Conquet F, Saga Y, Fukuda A, Guidepost neurons for the lateral olfactory tract: expression of metabotropic glutamate receptor 1 and innervation by glutamatergic olfactory bulb axons. Dev Neurobiol. 2012 Dec;72(12):1559-76

- Bellone C, Mameli M, Luscher C, In utero exposure to cocaine delays postnatal synaptic maturation of glutamatergic transmission in the VTA. Nat Neurosci. 2011 Nov;14(11):1439-46

- Yamasaki M, Miyazaki T, Azechi H, Abe M, Natsume R, Hagiwara T, Aiba A, Mishina M, Sakimura K, Watanabe M, Glutamate receptor delta2 is essential for input pathway-dependent regulation of synaptic AMPAR contents in cerebellar Purkinje cells. J Neurosci. 2011 Mar 2;31(9):3362-74

- Puliti A, Rossi PI, Caridi G, Corbelli A, Ikehata M, Armelloni S, Li M, Zennaro C, Conti V, Vaccari CM, Cassanello M, Calevo MG, Emionite L, Ravazzolo R, Rastaldi MP, Albuminuria and glomerular damage in mice lacking the metabotropic glutamate receptor 1. Am J Pathol. 2011 Mar;178(3):1257-69

- Zhang L, Alger BE, Enhanced endocannabinoid signaling elevates neuronal excitability in fragile X syndrome. J Neurosci. 2010 Apr 21;30(16):5724-9

- Le Vasseur M, Ran I, Lacaille JC, Selective induction of metabotropic glutamate receptor 1- and metabotropic glutamate receptor 5-dependent chemical long-term potentiation at oriens/alveus interneuron synapses of mouse hippocampus. Neuroscience. 2008 Jan 2;151(1):28-42

- Galik J, Youn DH, Kolaj M, Randic M, Involvement of group I metabotropic glutamate receptors and glutamate transporters in the slow excitatory synaptic transmission in the spinal cord dorsal horn. Neuroscience. 2008 Jul 17;154(4):1372-87

- Mouse Genome Informatics (MGI) and National Center for Biotechnology Information (NCBI), Mouse Gene Trap Data Load from dbGSS. Database Download. 2008;

- De Saint Jan D, Westbrook GL, Disynaptic amplification of metabotropic glutamate receptor 1 responses in the olfactory bu b. J Neurosci. 2007 Jan 3;27(1):132-40

- Sachs AJ, Schwendinger JK, Yang AW, Haider NB, Nystuen AM, The mouse mutants recoil wobbler and nmf373 represent a series of Grm1 mutations. Mamm Genome. 2007 Nov;18(11):749-56

- Heinbockel T, Laaris N, Ennis M, Metabotropic glutamate receptors in the main olfactory bulb drive granule cell-mediated inh bition. J Neurophysiol. 2007 Jan;97(1):858-70

- Nakao H, Nakao K, Kano M, Aiba A, Metabotropic glutamate receptor subtype-1 is essential for motor coordination in the adult cerebellum. Neurosci Res. 2007 Apr;57(4):538-43

- Fogal B, Li J, Lobner D, McCullough LD, Hewett SJ, System x(c)- activity and astrocytes are necessary for interleukin-1 beta-mediated hypoxic neuronal injury. J Neurosci. 2007 Sep 19;27(38):10094-105

- Conti V, Aghaie A, Cilli M, Martin N, Caridi G, Musante L, Candiano G, Castagna M, Fairen A, Ravazzolo R, Guenet JL, Puliti A, crv4, a mouse model for human ataxia associated with kyphoscoliosis caused by an mRNA splicing mutation of the metabotropic glutamate receptor 1 (Grm1). Int J Mol Med. 2006 Oct;18(4):593- 600

- Harris BS, Ward-Bailey P, Johnson KR, Bronson RT, Recoil Wobbler 4 Jackson, A New Remutation to Recoil Wobbler on Chromosome 10. MGI Direct Data Submission. 2006;

- The Australian Phenomics Facility at The Australian National University, Heritable mouse mutants from the ENU mutagenesis program at the Australian Phenomics Facility at The Australian National University. MGI Direct Data Submission. 2006-2014;

- Karst SY, Ward-Bailey PF, Donahue LR, Johnson KR, Davisson MT, Recoil Wobbler 3 Jackson, a remutation on proximal Chromosome 10. MGI Direct Data Submission. 2005;

- Bortolotto ZA, Collett VJ, Conquet F, Jia Z, van der Putten H, Collingridge GL, The regulation of hippocampal LTP by the molecular switch, a form of metaplasticity, requires mGlu5 receptors. Neuropharmacology. 2005;49 Suppl 1:13-25

- Deltagen Inc, NIH initiative supporting placement of Deltagen, Inc. mice into public repositories. MGI Direct Data Submission. 2005;

- Cook SA, Bronson RT, Davisson MT, Recoil Wobbler 2 Jackson, rcw<2J>, a new neurological mutation in the proximal region of mouse Chromosome 10. MGI Direct Data Submission. 2004;

- JAX Neuroscience Mutagenesis Facility, Heritable mouse mutants from JAX NMF ENU Mutagenesis Program. MGI Direct Data Submission. 2004-7;

- Sato M, Tabata T, Hashimoto K, Nakamura K, Nakao K, Katsuki M, Kitano J, Moriyoshi K, Kano M, Nakanishi S, Altered agonist sensitivity and desensitization of neuronal mGluR1 responses in knock-in mice by a single amino acid substitution at the PKC phosphorylation site. Eur J Neurosci. 2004 Aug;20(4):947-55

- Lapointe V, Morin F, Ratte S, Croce A, Conquet F, Lacaille JC, Synapse-specific mGluR1-dependent long-term potentiation in interneurones regulates mouse hippocampal inh bition. J Physiol. 2004 Feb 15;555(Pt 1):125-35

- Cook SA, Bronson RT, Davisson MT, A new neurological mutation in the proximal region of mouse Chromosome 10. Mouse Mutant Resources Web Site, The Jackson Laboratory, Bar Harbor, Maine. MGI Direct Data Submission. 2003;

- Stoop R, Conquet F, Pralong E, Determination of group I metabotropic glutamate receptor subtypes involved in the frequency of epileptiform activity in vitro using mGluR1 and mGluR5 mutant mice. Neuropharmacology. 2003 Feb;44(2):157-62

- Gubellini P, Saulle E, Centonze D, Costa C, Tropepi D, Bernardi G, Conquet F, Calabresi P, Corticostriatal LTP requires combined mGluR1 and mGluR5 activation. Neuropharmacology. 2003 Jan;44(1):8-16

- Brody SA, Conquet F, Geyer MA, Disruption of prepulse inhibition in mice lacking mGluR1. Eur J Neurosci. 2003 Dec;18(12):3361-6

- Neuroscience Mutagenesis Facility, Heritable mouse mutants from JAX NMF ENU Mutagenesis Program. MGI Direct Data Submission. 2003;

- Pollock PM, Cohen-Solal K, Sood R, Namkoong J, Martino JJ, Koganti A, Zhu H, Robbins C, Makalowska I, Shin SS, Marin Y, Roberts KG, Yudt LM, Chen A, Cheng J, Incao A, Pinkett HW, Graham CL, Dunn K, Crespo-Carbone SM, Mackason KR, Ryan KB, Sinsimer D, Goydos J, Reuhl KR, Eckhaus M, Meltzer PS, Pavan WJ, Trent JM, Chen S, Melanoma mouse model implicates metabotropic glutamate signaling in melanocytic neoplasia. Nat Genet. 2003 May;34(1):108-12

- Kishimoto Y, Fujimichi R, Araishi K, Kawahara S, Kano M, A ba A, Kirino Y, mGluR1 in cerebellar Purkinje cells is required for normal association of temporally contiguous stimuli in classical conditioning. Eur J Neurosci. 2002 Dec;16(12):2416-24

- Chuang SC, Zhao W, Young SR, Conquet F, Bianchi R, Wong RK, Activation of group I mGluRs elicits different responses in murine CA1 and CA3 pyramidal cells. J Physiol. 2002 May 15;541(Pt 1):113-21

- Shutoh F, Katoh A, Kitazawa H, Aiba A, Itohara S, Nagao S, Loss of adaptability of horizontal optokinetic response eye movements in mGluR1 knockout mice. Neurosci Res. 2002 Feb;42(2):141-5

- Tabata T, Aiba A, Kano M, Extracellular calcium controls the dynamic range of neuronal metabotropic glutamate receptor responses. Mol Cell Neurosci. 2002 May;20(1):56-68

- Gubellini P, Saulle E, Centonze D, Bonsi P, Pisani A, Bernardi G, Conquet F, Calabresi P, Selective involvement of mGlu1 receptors in corticostriatal LTD. Neuropharmacology. 2001 Jun;40(7):839-46

- Mao L, Conquet F, Wang JQ, Augmented motor activity and reduced striatal preprodynorphin mRNA induction in response to acute amphetamine administration in metabotropic glutamate receptor 1 knockout mice. Neuroscience. 2001;106(2):303-12

- Hashimoto K, Ichikawa R, Takechi H, Inoue Y, A ba A, Sakimura K, Mishina M, Hashikawa T, Konnerth A, Watanabe M, Kano M, Roles of glutamate receptor delta 2 subunit (GluRdelta 2) and metabotropic glutamate receptor subtype 1 (mGluR1) in climbing f ber synapse elimination during postnatal cerebellar development. J Neurosci. 2001 Dec 15;21(24):9701-12

- Pisani A, Gubellini P, Bonsi P, Conquet F, Picconi B, Centonze D, Bernardi G, Calabresi P, Metabotropic glutamate receptor 5 mediates the potentiation of N-methyl-D-aspartate responses in medium spiny striatal neurons. Neuroscience. 2001;106(3):579-87

- Ichise T, Kano M, Hashimoto K, Yanagihara D, Nakao K, Shigemoto R, Katsuki M, Aiba A, mGluR1 in cerebellar Purkinje cells essential for long-term depression, synapse elimination, and motor coordination. Science. 2000 Jun 9;288(5472):1832-5

- Sistiaga A, Herrero I, Conquet F, Sanchez-Prieto J, The metabotropic glutamate receptor 1 is not involved in the facilitation of glutamate release in cerebrocortical nerve terminals. Neuropharmacology. 1998 Dec;37(12):1485-92

- Levenes C, Daniel H, Jaillard D, Conquet F, Crepel F, Incomplete regression of multiple climbing f bre innervation of cerebellar Purkinje cells in mGLuR1 mutant mice. Neuroreport. 1997 Jan 20;8(2):571-4

- Bordi F, Reggiani A, Conquet F, Regulation of synaptic plasticity by mGluR1 studied in vivo in mGluR1 mutant mice. Brain Res. 1997 Jun 27;761(1):121-6

- Kano M, Hashimoto K, Kurihara H, Watanabe M, Inoue Y, Aiba A , Tonegawa S, Persistent multiple climbing f ber innervation of cerebellar Purkinje cells in mice lacking mGluR1. Neuron. 1997 Jan;18(1):71-9

- Chen S, Zhu H, Wetzel WJ, Phi bert MA, Spontaneous melanocytosis in transgenic mice. J Invest Dermatol. 1996 May;106(5):1145-51

- Aiba A, Chen C, Herrup K, Rosenmund C, Stevens CF, Tonegawa S, Reduced hippocampal long-term potentiation and context-specific deficit in associative learning in mGluR1 mutant mice. Cell. 1994 Oct 21;79(2):365-75

- Conquet F, Bashir ZI, Davies CH, Daniel H, Ferraguti F, Bordi F, Franz-Bacon K, Reggiani A, Matarese V, Conde F, Collingridge GL, Crepel F, Motor deficit and impairment of synaptic plasticity in mice lacking mGluR1 [see comments]. Nature. 1994 Nov 17;372(6503):237-43

- Aiba A, Kano M, Chen C, Stanton ME, Fox GD, Herrup K, Zwingman TA, Tonegawa S, Deficient cerebellar long-term depression and impaired motor learning in mGluR1 mutant mice. Cell. 1994 Oct 21;79(2):377-88

- Colon-Teicher L, Wise LS, Martino JJ, Baskin L, Sakoulas G, Pollack RE, Chen S, Genomic sequences capable of committing mouse and rat fibroblasts to adipogenesis. Nucleic Acids Res. 1993 May 11;21(9):2223-8

GRM2

Knockout references

http://www.mousephenotype.org/data/genes/MGI:1351339
http://www.informatics.jax.org/marker/MGI:1351339

Phenotype references

- Battaglia G, Riozzi B, Bucci D, Di Menna L, Molinaro G, Pallottino S, Nicoletti F, Bruno V, Activation of mGlu3 metabotropic glutamate receptors enhances GDNF and GLT-1 formation in the spinal cord and rescues motor neurons in the SOD-1 mouse model of amyotrophic lateral sclerosis. Neurobiol Dis. 2015 Feb;74:126-36

- Nasca C, Xenos D, Barone Y, Caruso A, Scaccianoce S, Matrisciano F, Battaglia G, Mathe AA, Pittaluga A, Lionetto L, Simmaco M, Nicoletti F, L-acetylcarnitine causes rapid antidepressant effects through the epigenetic induction of mGlu2 receptors. Proc Natl Acad Sci U S A. 2013 Mar 19;110(12):4804-9

- Zhou Z, Karlsson C, Liang T, Xiong W, Kimura M, Tapocik JD, Yuan Q, Barbier E, Feng A, Flanigan M, Augier E, Enoch MA, Hodgkinson CA, Shen PH, Lovinger DM, Edenberg HJ, Heilig M, Goldman D, Loss of metabotropic glutamate receptor 2 escalates alcohol consumption. Proc Natl Acad Sci U S A. 2013 Oct 15;110(42):16963-8

- Lane TA, Boerner T, Bannerman DM, Kew JN, Tunbridge EM, Sharp T, Harrison PJ, Decreased striatal dopamine in group II metabotropic glutamate receptor (mGlu2/mGlu3) double knockout mice. BMC Neurosci. 2013;14:102

- Fribourg M, Moreno JL, Holloway T, Provasi D, Baki L, Mahajan R, Park G, Adney SK, Hatcher C, Eltit JM, Ruta JD, Albizu L, Li Z, Umali A, Shim J, Fabiato A, Mackerell AD Jr, Brezina V, Sealfon SC, Filizola M, Gonzalez-Maeso J, Logothetis DE, Decoding the Signaling of a GPCR Heteromeric Complex Reveals a Unifying Mechanism of Action of Antipsychotic Drugs. Cell. 2011 Nov 23;147(5):1011-23

- Ster J, Mateos JM, Grewe BF, Coiret G, Corti C, Corsi M, Helmchen F, Gerber U, Enhancement of CA3 hippocampal network activity by activation of group II metabotropic glutamate receptors. Proc Natl Acad Sci U S A. 2011 Jun 14;108(24):9993-7

- Moreno JL, Holloway T, Albizu L, Sealfon SC, Gonzalez-Maeso J, Metabotropic glutamate mGlu2 receptor is necessary for the pharmacological and behavioral effects induced by hallucinogenic 5-HT2A receptor agonists. Neurosci Lett. 2011 Apr 15;493(3):76-9

- Skarnes WC, Rosen B, West AP, Koutsourakis M, Bushell W, Iyer V, Mujica AO, Thomas M, Harrow J, Cox T, Jackson D, Severin J, Biggs P, Fu J, Nefedov M, de Jong PJ, Stewart AF, Bradley A, A conditional knockout resource for the genome-wide study of mouse gene function. Nature. 2011 Jun 16;474(7351):337- 42

- Linden AM, Johnson BG, Trokovic N, Korpi ER, Schoepp DD, Use of MGLUR2 and MGLUR3 knockout mice to explore in vivo receptor specificity of the MGLUR2/3 selective antagonist LY341495. Neuropharmacology. 2009 Aug;57(2):172-82

- Corti C, Battaglia G, Molinaro G, Riozzi B, Pittaluga A, Corsi M, Mugnaini M, Nicoletti F, Bruno V, The use of knock-out mice unravels distinct roles for mGlu2 and mGlu3 metabotropic glutamate receptors in mechanisms of neurodegeneration/neuroprotection. J Neurosci. 2007 Aug 1;27(31):8297-308

- Linden AM, Baez M, Bergeron M, Schoepp DD, Effects of mGlu2 or mGlu3 receptor deletions on mGlu2/3 receptor agonist (LY354740)-induced brain c-Fos expression: specific roles for mGlu2 in the amygdala and subcortical nuclei, and mGlu3 in the hippocampus. Neuropharmacology. 2006 Aug;51(2):213-28

- Morishima Y, Miyakawa T, Furuyashiki T, Tanaka Y, Mizuma H, Nakanishi S, Enhanced cocaine responsiveness and impaired motor coordination in metabotropic glutamate receptor subtype 2 knockout mice. Proc Natl Acad Sci U S A. 2005 Mar 15;102(11):4170-5

- Linden AM, Shannon H, Baez M, Yu JL, Koester A, Schoepp DD, Anxiolytic-like activity of the mGLU2/3 receptor agonist LY354740 in the elevated plus maze test is disrupted in metabotropic glutamate receptor 2 and 3 knock-out mice. Psychopharmacology (Berl). 2005 Apr;179(1):284-91

- Higgins GA, Ballard TM, Kew JN, Richards JG, Kemp JA, Adam G, Woltering T, Nakanishi S, Mutel V, Pharmacological manipulation of mGlu2 receptors influences cognitive performance in the rodent. Neuropharmacology. 2004 Jun;46(7):907-17

- Renger JJ, Hartman KN, Tsuchimoto Y, Yokoi M, Nakanishi S, Hensch TK, Experience-dependent plasticity without long-term depression by type 2 metabotropic glutamate receptors in developing visual cortex. Proc Natl Acad Sci U S A. 2002 Jan 22;99(2):1041-6

- Kew JN, Pflimlin MC, Kemp JA, Mutel V, Differential regulation of synaptic transmission by mGlu2 and mGlu3 at the perforant path inputs to the dentate gyrus and CA1 revealed in mGlu2 -/- mice. Neuropharmacology. 2002 Aug;43(2):215-21

- Yokoi M, Kobayashi K, Manabe T, Takahashi T, Sakaguchi I, Katsuura G, Shigemoto R, Ohishi H, Nomura S, Nakamura K, Nakao K, Katsuki M, Nakanishi S, Impairment of hippocampal mossy f ber LTD in mice lacking mGluR2. Science. 1996 Aug 2;273(5275):645-7

GRM3

Knockout references

http://www.mousephenotype.org/data/genes/MGI:1351340
http://www.informatics.jax.org/marker/MGI:1351340

Phenotype references

- Battaglia G, Riozzi B, Bucci D, Di Menna L, Molinaro G, Pallottino S, Nicoletti F, Bruno V, Activation of mGlu3 metabotropic glutamate receptors enhances GDNF and GLT-1 formation in the spinal cord and rescues motor neurons in the SOD-1 mouse model of amyotrophic lateral sclerosis. Neurobiol Dis. 2015 Feb;74:126-36

- Lane TA, Boerner T, Bannerman DM, Kew JN, Tunbridge EM, Sharp T, Harrison PJ, Decreased striatal dopamine in group II metabotropic glutamate receptor (mGlu2/mGlu3) double knockout mice. BMC Neurosci. 2013;14:102

- Skarnes WC, Rosen B, West AP, Koutsourakis M, Bushell W, Iyer V, Mujica AO, Thomas M, Harrow J, Cox T, Jackson D, Severin J, Biggs P, Fu J, Nefedov M, de Jong PJ, Stewart AF, Bradley A, A conditional knockout resource for the genome-wide study of mouse gene function. Nature. 2011 Jun 16;474(7351):337- 42

- Linden AM, Johnson BG, Trokovic N, Korpi ER, Schoepp DD, Use of MGLUR2 and MGLUR3 knockout mice to explore in vivo receptor specificity of the MGLUR2/3 selective antagonist LY341495. Neuropharmacology. 2009 Aug;57(2):172-82

- Corti C, Battaglia G, Molinaro G, Riozzi B, Pittaluga A, Corsi M, Mugnaini M, Nicoletti F, Bruno V, The use of knock-out mice unravels distinct roles for mGlu2 and mGlu3 metabotropic glutamate receptors in mechanisms of neurodegeneration/neuroprotection. J Neurosci. 2007 Aug 1;27(31):8297-308

- Linden AM, Baez M, Bergeron M, Schoepp DD, Effects of mGlu2 or mGlu3 receptor deletions on mGlu2/3 receptor agonist (LY354740)-induced brain c-Fos expression: specific roles for mGlu2 in the amygdala and subcortical nuclei, and mGlu3 in the hippocampus. Neuropharmacology. 2006 Aug;51(2):213-28

- Linden AM, Shannon H, Baez M, Yu JL, Koester A, Schoepp DD, Anxiolytic-like activity of the mGLU2/3 receptor agonist LY354740 in the elevated plus maze test is disrupted in metabotropic glutamate receptor 2 and 3 knock-out mice. Psychopharmacology (Berl). 2005 Apr;179(1):284-91

GRM4

Knockout references

http://www.mousephenotype.org/data/genes/MGI:1351341
http://www.informatics.jax.org/marker/MGI:1351341

Phenotype references

- Davis MJ, Iancu OD, Acher FC, Stewart BM, Eiwaz MA, Duvoisin RM, Raber J, Role of mGluR4 in acquisition of fear learning and memory. Neuropharmacology. 2013 Mar;66:365-72

- 24285900Vilar B, Busserolles J, Ling B, Laffray S, Ulmann L, Malhaire F, Chapuy E, Aissouni Y, Etienne M, Bourinet E, Acher F, Pin JP, Eschalier A, Goudet C, Alleviating pain hypersensitivity through activation of type 4 metabotropic glutamate receptor. J Neurosci. 2013 Nov 27;33(48):18951-65

- Iscru E, Goddyn H, Ahmed T, Callaerts-Vegh Z, D'Hooge R, Balschun D, Improved spatial learning is associated with increased hippocampal but not prefrontal long-term potentiation in mGluR4 knockout mice. Genes Brain Behav. 2013 Aug;12(6):615-25

- Flachs P, Mihola O, Simecek P, Gregorova S, Schimenti JC, Matsui Y, Baudat F, de Massy B, Pialek J, Forejt J, Trachtulec Z, Interallelic and intergenic incompatibilities of the Prdm9 (Hst1) gene in mouse hybrid sterility. PLoS Genet. 2012;8(11):e1003044

- Davis MJ, Haley T, Duvoisin RM, Raber J, Measures of anxiety, sensorimotor function, and memory in male and female mGluR4(-)/(-) mice. Behav Brain Res. 2012 Apr 1;229(1):21-8

- Fallarino F, Volpi C, Fazio F, Notartomaso S, Vacca C, Busceti C, Bicciato S, Battaglia G, Bruno V, Puccetti P, Fioretti MC, Nicoletti F, Grohmann U, Di Marco R, Metabotropic glutamate receptor-4 modulates adaptive immunity and restrains neuroinflammation. Nat Med. 2010 Aug;16(8):897-902

- Moritz A, Scheschonka A, Beckhaus T, Karas M, Betz H, Metabotropic glutamate receptor 4 interacts with microtubule-associated protein 1B. Biochem Biophys Res Commun. 2009 Dec 4;390(1):82-6

- Hansen GM, Markesich DC, Burnett MB, Zhu Q, Dionne KM, Richter LJ, Finnell RH, Sands AT, Zambrowicz BP, Abuin A, Large-scale gene trapping in C57BL/6N mouse embryonic stem cells. Genome Res. 2008 Oct;18(10):1670-9

- Pitsch J, Schoch S, Gueler N, Flor PJ, van der Putten H, Becker AJ, Functional role of mGluR1 and mGluR4 in pilocarpine-induced temporal lobe epilepsy. Neurobiol Dis. 2007 Jun;26(3):623-33

- Battaglia G, Busceti CL, Molinaro G, Biagioni F, Traficante A, Nicoletti F, Bruno V, Pharmacological activation of mGlu4 metabotropic glutamate receptors reduces nigrostriatal degeneration in mice treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. J Neurosci. 2006 Jul 5;26(27):7222-9

- Wang X, Ai J, Hampson DR, Snead OC 3rd, Altered glutamate and GABA release within thalamocortical circuitry in metabotropic glutamate receptor 4 knockout mice. Neuroscience. 2005;134(4):1195-203

- Howell GR, Bergstrom RA, Munroe RJ, Masse J, Schimenti JC, Identification of a cryptic lethal mutation in the mouse t(w73) haplotype. Genet Res. 2004 Dec;84(3):153-9

- Blednov YA, Walker D, Osterndorf-Kahanek E, Harris RA, Mice lacking metabotropic glutamate receptor 4 do not show the motor stimulatory effect of ethanol. Alcohol. 2004 Oct-Nov;34(2-3):251-9

- Bergstrom DE, Bergstrom RA, Munroe RJ, Lee BK, Browning VL, You Y, Eicher EM, Schimenti JC, Overlapping deletions spanning the proximal two-thirds of the mouse t complex. Mamm Genome. 2003 Dec;14(12):817-29

- Bruno V, Battaglia G, Ksiazek I, van der Putten H, Catania MV, Giuffrida R, Lukic S, Leonhardt T, Inderbitzin W, Gasparini F, Kuhn R, Hampson DR, Nicoletti F, Flor PJ, Selective activation of mGlu4 metabotropic glutamate receptors is protective against excitotoxic neuronal death. J Neurosci. 2000 Sep 1;20(17):6413-20

- Snead OC 3rd, Banerjee PK, Burnham M, Hampson D, Modulation of absence seizures by the GABA(A) receptor: a critical rolefor metabotropic glutamate receptor 4 (mGluR4). J Neurosci. 2000 Aug 15;20(16):6218-24

- Gerlai R, Roder JC, Hampson DR, Altered spatial learning and memory in mice lacking the mGluR4 subtype of metabotropic glutamate receptor. Behav Neurosci. 1998 Jun;112(3):525-32

- Pekhletski R, Gerlai R, Overstreet LS, Huang XP, Agopyan N, Slater NT, Abramow-Newerly W, Roder JC, Hampson DR, Impaired cerebellar synaptic plasticity and motor performance in mice lacking the mGluR4 subtype of metabotropic glutamate receptor. J Neurosci. 1996 Oct 15;16(20):6364-73

GRM5

Knockout references

http://www.mousephenotype.org/data/genes/MGI:1351342
http://www.informatics.jax.org/marker/MGI:1351342

Phenotype references

- Shin S, Kwon O, Kang JI, Kwon S, Oh S, Choi J, Kim CH, Kim DG, mGluR5 in the nucleus accumbens is critical for promoting resilience to chronic stress. Nat Neurosci. 2015 Jul;18(7):1017-24

- Xu J, Antion MD, Nomura T, Kraniotis S, Zhu Y, Contractor A, Hippocampal metaplasticity is required for the formation of temporal associative memories. J Neurosci. 2014 Dec 10;34(50):16762-73

- Ribeiro FM, Devries RA, Hamilton A, Guimaraes IM, Cregan SP, Pires RG, Ferguson SS, Metabotropic glutamate receptor 5 knockout promotes motor and biochemical alterations in a mouse model of Huntington's disease. Hum Mol Genet. 2014 Apr15;23(8):2030-42

- Sidorov MS, Krueger DD, Taylor M, Gisin E, Osterweil EK, Bear MF, Extinction of an instrumental response: a cognitive behavioral assay in Fmr1 knockout mice. Genes Brain Behav. 2014 Jun;13(5):451-8

- Vogt MA, Luoni A, Filipovic D, Lima-Ojeda JM, Pfeiffer N, Gasparini F, Riva MA, Gass P, Significant increase in anxiety during aging in mGlu5 receptor knockout mice. Behav Brain Res. 2013 Mar 15;241:27-31

- Park JM, Hu JH, Milshteyn A, Zhang PW, Moore CG, Park S, Datko MC, Domingo RD, Reyes CM, Wang XJ, Etzkorn FA, Xiao B, Szumlinski KK, Kern D, Linden DJ, Worley PF, A prolyl-isomerase mediates dopamine-dependent plasticity and cocaine motor sensitization. Cell. 2013 Aug 1;154(3):637-50

- Xiao XL, Ma DL, Wu J, Tang FR, Metabotropic glutamate receptor 5 (mGluR5) regulates proliferation and differentiation of neuronal progenitors in the developmental hippocampus. Brain Res. 2013 Feb 1;1493:1-12

- Um JW, Kaufman AC, Kostylev M, Heiss JK, Stagi M, Takahashi H, Kerrisk ME, Vortmeyer A, Wisniewski T, Koleske AJ, Gunther EC, Nygaard HB, Strittmatter SM, Metabotropic glutamate receptor 5 is a coreceptor for Alzheimer abeta oligomer bound to cellular prion protein. Neuron. 2013 Sep 4;79(5):887-902

- Chesworth R, Brown RM, Kim JH, Lawrence AJ, The metabotropic glutamate 5 receptor modulates extinction and reinstatement of methamphetamine-seeking in mice. PLoS One. 2013;8(7):e68371

- Jew CP, Wu CS, Sun H, Zhu J, Huang JY, Yu D, Justice NJ, Lu HC, mGluR5 ablation in cortical glutamatergic neurons increases novelty-induced locomotion. PLoS One. 2013;8(8):e70415

- Chen CC, Lu HC, Brumberg JC, mGluR5 knockout mice display increased dendritic spine densities. Neurosci Lett. 2012 Aug 22;524(1):65-8

- Hu JH, Yang L, Kammermeier PJ, Moore CG, Brakeman PR, Tu J, Yu S, Petralia RS, Li Z, Zhang PW, Park JM, Dong X, Xiao B, Worley PF, Preso1 dynamically regulates group I metabotropic glutamate receptors. Nat Neurosci. 2012 Jun;15(6):836-44

- Hays SA, Huber KM, Gibson JR, Altered Neocortical Rhythmic Activity States in Fmr1 KO Mice Are Due to Enhanced mGluR5 Signaling and Involve Changes in Excitatory Circuitry. J Neurosci. 2011 Oct 5;31(40):14223-34

- Zhang L, Alger BE, Enhanced endocannabinoid signaling elevates neuronal excitability in fragile X syndrome. J Neurosci. 2010 Apr 21;30(16):5724-9

- Kolber BJ, Montana MC, Carrasquillo Y, Xu J, Heinemann SF, Muglia LJ, Gereau RW 4th, Activation of metabotropic glutamate receptor 5 in the amygdala modulates pain-like behavior. J Neurosci. 2010 Jun 16;30(24):8203-13

- Olsen CM, Childs DS, Stanwood GD, Winder DG, Operant sensation seeking requires metabotropic glutamate receptor 5 (mGluR5). PLoS One. 2010;5(11):e15085

- Ballester-Rosado CJ, A bright MJ, Wu CS, Liao CC, Zhu J, Xu J, Lee LJ, Lu HC, mGluR5 in cortical excitatory neurons exerts both cell-autonomous and -nonautonomous influences on cortical somatosensory circuit formation. J Neurosci. 2010 Dec 15;30(50):16896-909

- Downing C, Marks MJ, Larson C, Johnson TE, The metabotropic glutamate receptor subtype 5 mediates sensitivity to the sedative properties of ethanol. Pharmacogenet Genomics. 2010 Sep;20(9):553-64

- Ribeiro FM, Paquet M, Ferreira LT, Cregan T, Swan P, Cregan SP, Ferguson SS, Metabotropic glutamate receptor-mediated cell signaling pathways are altered in a mouse model of Huntington's disease. J Neurosci. 2010 Jan 6;30(1):316-24

- Cozzoli DK, Goulding SP, Zhang PW, Xiao B, Hu JH, Ary AW, Obara I, Rahn A, Abou-Ziab H, Tyrrel B, Marini C, Yoneyama N, Metten P, Snelling C, Dehoff MH, Crabbe JC, Finn DA, Klugmann M, Worley PF, Szumlinski KK, Binge drinking upregulates accumbens mGluR5-Homer2-PI3K signaling: functional implications for alcoholism. J Neurosci. 2009 Jul 8;29(27):8655-68

- She WC, Quairiaux C, Albright MJ, Wang YC, Sanchez DE, Chang PS, We ker E, Lu HC, Roles of mGluR5 in synaptic function and plasticity of the mouse thalamocortical pathway. Eur J Neurosci. 2009 Apr;29(7):1379-96

- Sibaev A, Yuce B, Kemmer M, Van Nassauw L, Broedl U, Allescher HD, Goke B, Timmermans JP, Storr M, Cannabinoid-1 (CB1) receptors regulate colonic propulsion by acting at motor neurons within the ascending motor pathways in mouse colon. Am J Physiol Gastrointest Liver Physiol. 2009 Jan;296(1):G119-28

- Sidiropoulou K, Lu FM, Fowler MA, Xiao R, Phillips C, Ozkan ED, Zhu MX, White FJ, Cooper DC, Dopamine modulates an mGluR5-mediated depolarization underlying prefrontal persistent activity. Nat Neurosci. 2009 Feb;12(2):190-9

- Xu J, Zhu Y, Contractor A, Heinemann SF, mGluR5 has a critical role in inh bitory learning. J Neurosci. 2009 Mar 25;29(12):3676-84

GRM6

Knockout references

http://www.mousephenotype.org/data/genes/MGI:1351343
http://www.informatics.jax.org/marker/MGI:1351343

Phenotype references

- Ray TA, Heath KM, Hasan N, Noel JM, Samuels IS, Martemyanov KA, Peachey NS, McCall MA, Gregg RG, GPR179 is required for high sensitivity of the mGluR6 signaling cascade in depolarizing bipolar cells. J Neurosci. 2014 Apr 30;34(18):6334-43

- Klooster J, van Genderen MM, Yu M, Florijn RJ, Riemslag FC, Bergen AA, Gregg RG, Peachey NS, Kamermans M, Ultrastructural localization of GPR179 and the impact of mutant forms on retinal function in CSNB1 patients and a mouse model. Invest Ophthalmol Vis Sci. 2013;54(10):6973-81

- Dhingra A, Ramakrishnan H, Neinstein A, Fina ME, Xu Y, Li J, Chung DC, Lyubarsky A, Vardi N, Gbeta3 is required for normal light on responses and synaptic maintenance. J Neurosci. 2012 Aug 15;32(33):11343-55

- Peachey NS, Pearring JN, Bojang P Jr, Hirschtritt ME, Sturgill-Short G, Ray TA, Furukawa T, Koike C, Goldberg AF, Shen Y, McCall MA, Nawy S, Nishina PM, Gregg RG, Depolarizing bipolar cell dysfunction due to a Trpm1 point mutation. J Neurophysiol. 2012 Nov;108(9):2442-51

- Xu Y, Dhingra A, Fina ME, Koike C, Furukawa T, Vardi N, mGluR6 deletion renders the TRPM1 channel in retina inactive. J Neurophysiol. 2012 Feb;107(3):948-57

- Thompson S, Stasheff SF, Hernandez J, Nylen E, East JS, Kardon RH, Pinto LH, Mullins RF, Stone EM, Different inner retinal pathways mediate rod-cone input in irradiance detection for the pupillary light reflex and regulation of behavioral state in mice. Invest Ophthalmol Vis Sci. 2011 Jan;52(1):618-23

- Won J, Shi LY, Hicks W, Wang J, Hurd R, Naggert JK, Chang B, Nishina PM, Mouse model resources for vision research. J Ophthalmol. 2011;2011:391384

- Vardi T, Fina M, Zhang L, Dhingra A, Vardi N, mGluR6 transcripts in non-neuronal tissues. J Histochem Cytochem. 2011 Dec;59(12):1076-86

- Skarnes WC, Rosen B, West AP, Koutsourakis M, Bushell W, Iyer V, Mujica AO, Thomas M, Harrow J, Cox T, Jackson D, Severin J, Biggs P, Fu J, Nefedov M, de Jong PJ, Stewart AF, Bradley A, A conditional knockout resource for the genome-wide study of mouse gene function. Nature. 2011 Jun 16;474(7351):337- 42

- Cao Y, Masuho I, Okawa H, Xie K, Asami J, Kammermeier PJ, Maddox DM, Furukawa T, Inoue T, Sampath AP, Martemyanov KA, Retina-specific GTPase accelerator RGS11/G beta 5S/R9AP is a constitutive heterotrimer selectively targeted to mGluR6 in ON-bipolar neurons. J Neurosci. 2009 Jul 22;29(29):9301-13

- Koyasu T, Kondo M, Miyata K, Ueno S, Miyata T, Nishizawa Y, Terasaki H, Photopic Electroretinograms of mGluR6-Deficient Mice. Curr Eye Res. 2008 Jan;33(1):91-9

- Maddox DM, Vessey KA, Yarbrough GL, Invergo BM, Cantrell DR, Inayat S, Balannik V, Hicks WL, Hawes NL, Byers S, Smith RS, Hurd R, Howell D, Gregg RG, Chang B, Naggert JK, Troy JB, Pinto LH, Nishina PM, McCall MA, Allelic variance between GRM6 mutants, Grm6nob3 and Grm6nob4 results in differences in retinal ganglion cell visual responses. J Physiol. 2008 Sep 15;586(Pt 18):4409-24

- Morgans CW, Wensel TG, Brown RL, Perez-Leon JA, Bearnot B, Duvoisin RM, Gbeta5-RGS complexes co-localize with mGluR6 in retinal ON-bipolar cells. Eur J Neurosci. 2007 Nov;26(10):2899-905

- Pinto LH, Vitaterna MH, Shimomura K, Siepka SM, Balannik V, McDearmon EL, Omura C, Lumayag S, Invergo BM, Glawe B, Cantrell DR, Inayat S, Olvera MA, Vessey KA, McCall MA, Maddox D, Morgans CW, Young B, Pletcher MT, Mullins RF, Troy JB, Takahashi JS, Generation, identification and functional characterization of the nob4 mutation of Grm6 in the mouse. Vis Neurosci. 2007 Jan-Feb;24(1):111-23

- Renteria RC, Tian N, Cang J, Nakanishi S, Stryker MP, Copenhagen DR, Intrinsic ON responses of the retinal OFF pathway are suppressed by the ON pathway. J Neurosci. 2006 Nov 15;26(46):11857-69

- Chang B, Hawes NL, Hurd RE, Wang J, Howell D, Davisson MT, Roderick TH, Nusinowitz S, Heckenlively JR, Mouse models of ocular diseases. Vis Neurosci. 2005 Sep-Oct;22(5):587-93

- Takao M, Morigiwa K, Sasaki H, Miyoshi T, Shima T, Nakanishi S, Nagai K, Fukuda Y, Impaired behavioral suppression by light in metabotropic glutamate receptor subtype 6-deficient mice. Neuroscience. 2000;97(4):779-87

- Tagawa Y, Sawai H, Ueda Y, Tauchi M, Nakanishi S, Immunohistological studies of metabotropic glutamate receptor subtype 6-deficient mice show no abnormality of retinal cell organization and ganglion cell maturation. J Neurosci. 1999 Apr 1;19(7):2568-79

- Sugihara H, Inoue T, Nakanishi S, Fukuda Y, A late ON response remains in visual response of the mGluR6-deficient mouse. Neurosci Lett. 1997 Sep 19;233(2-3):137-40

- Lyon MF, Rastan S, Brown SDM, in Lyon MF, Rastan S, Brown SDM (eds), Genetic Variants and Strains of the Laboratory Mouse. 3rd Edition. 1996Oxford: Oxford University Press.

- Masu M, Iwakabe H, Tagawa Y, Miyoshi T, Yamashita M, Fukuda Y, Sasaki H, Hiroi K, Nakamura Y, Shigemoto R, et al, Specific deficit of the ON response in visual transmission by targeted disruption of the mGluR6 gene. Cell. 1995 Mar 10;80(5):757-65

- Roderick TH, Using inversions to detect and study recessive lethals and detrimentals in mice, in de Serres FJ, Sheridan W (eds), Utilization of Mammalian Specific Locus Studies in Hazard Evaluation and Estimation of Genetic Risk. 1983:135-67. Plenum Publishing Corp..

- Roderick TH, Chromosomal inversions in studies of mammalian mutagenesis. Genetics. 1979 May;92(1 Pt 1 Suppl):s121-6

GRM7

Knockout references

http://www.mousephenotype.org/data/genes/MGI:1351344

http://www.informatics.jax.org/marker/MGI:1351344

Phenotype references

- Skarnes WC, Rosen B, West AP, Koutsourakis M, Bushell W, Iyer V, Mujica AO, Thomas M, Harrow J, Cox T, Jackson D, Severin J, Biggs P, Fu J, Nefedov M, de Jong PJ, Stewart AF, Bradley A, A conditional knockout resource for the genome-wide study of mouse gene function. Nature. 2011 Jun 16;474(7351):337- 42

- Zhang CS, Bertaso F, Eulenburg V, Lerner-Natoli M, Herin GA, Bauer L, Bockaert J, Fagni L, Betz H, Scheschonka A, Knock-in mice lacking the PDZ-ligand motif of mGluR7a show impaired PKC-dependent autoinh bition of glutamate release, spatial working memory deficits, and increased susceptibility to pentylenetetrazol. J Neurosci. 2008 Aug 20;28(34):8604-14

- Goddyn H, Callaerts-Vegh Z, Stroobants S, Dir kx T, Vansteenwegen D, Hermans D, van der Putten H, D'Hooge R, Deficits in acquisition and extinction of conditioned responses in mGluR7 knockout mice. Neurobiol Learn Mem. 2008 Jul;90(1):103-11

- Hansen GM, Markesich DC, Burnett MB, Zhu Q, Dionne KM, Richter LJ, Finnell RH, Sands AT, Zambrowicz BP, Abuin A, Large-scale gene trapping in C57BL/6N mouse embryonic stem cells. Genome Res. 2008 Oct;18(10):1670-9

- Callaerts-Vegh Z, Beckers T, Ball SM, Baeyens F, Callaerts PF, F Cryan J, Molnar E, D'Hooge R, Concomitant deficits in working memory and fear extinction are functionally dissociated from reduced anxiety in metabotropic glutamate receptor 7-deficient mice. J Neurosci. 2006 Jun 14;26(24):6573-82

- Akeson EC, Donahue LR, Beamer WG, Shultz KL, Ackert-Bicknell C, Rosen CJ, Corrigan J, Davisson MT, Chromosomal inversion discovered in C3H/HeJ mice. Genomics. 2006 Feb;87(2):311-3

- Bortolotto ZA, Collett VJ, Conquet F, Jia Z, van der Putten H, Collingridge GL, The regulation of hippocampal LTP by the molecular switch, a form of metaplasticity, requires mGlu5 receptors. Neuropharmacology. 2005;49 Suppl 1:13-25

- Holscher C, Schmid S, Pilz PK, Sansig G, van der Putten H, Plappert CF, Lack of the metabotropic glutamate receptor subtype 7 selectively modulates Theta rhythm and working memory. Learn Mem. 2005 Sep-Oct;12(5):450-5

- Holscher C, Schmid S, Pilz PK, Sansig G, van der Putten H, Plappert CF, Lack of the metabotropic glutamate receptor subtype 7 selectively impairs short-term working memory but not long-term memory. Behav Brain Res. 2004 Oct 5;154(2):473-81

- Cryan JF, Kelly PH, Ne jt HC, Sansig G, Flor PJ, van Der Putten H, Antidepressant and anxiolytic-like effects in mice lacking the group III metabotropic glutamate receptor mGluR7. Eur J Neurosci. 2003 Jun;17(11):2409-17

- Akeson EC, Chromosomal inversion discovered in C3H/HeJ mice. JAX Notes. 2003;491:15

- Sansig G, Bushell TJ, Clarke VR, Rozov A, Burnashev N, Portet C, Gasparini F, Schmutz M, Klebs K, Shigemoto R, Flor PJ, Kuhn R, Knoepfel T, Schroeder M, Hampson DR, Collett VJ, Zhang C, Duvoisin RM, Collingridge GL, van Der Putten H, Increased seizure susceptibility in mice lacking metabotropic glutamate receptor 7. J Neurosci. 2001 Nov 15;21(22):8734-45

- Masugi M, Yokoi M, Shigemoto R, Muguruma K, Watanabe Y, Sansig G, van der Putten H, Nakanishi S, Metabotropic glutamate receptor subtype 7 ablation causes deficit in fear response and conditioned taste aversion. J Neurosci. 1999 Feb 1;19(3):955-63

GRM8

Knockout references

http://www.mousephenotype.org/data/genes/MGI:1351345
http://www.informatics.jax.org/marker/MGI:1351345

Phenotype references

- Skarnes WC, Rosen B, West AP, Koutsourakis M, Bushell W, Iyer V, Mujica AO, Thomas M, Harrow J, Cox T, Jackson D, Severin J, Biggs P, Fu J, Nefedov M, de Jong PJ, Stewart AF, Bradley A, A conditional knockout resource for the genome-wide study of mouse gene function. Nature. 2011 Jun 16;474(7351):337- 42

- Quraishi S, Reed BT, Duvoisin RM, Taylor WR, Selective activation of mGluR8 receptors modulates retinal ganglion cell light responses. Neuroscience. 2010 Mar 31;166(3):935-41

- Fendt M, Burki H, Imobersteg S, van der Putten H, McAllister K, Leslie JC, Shaw D, Holscher C, The effect of mGlu8 deficiency in animal models of psychiatric diseases. Genes Brain Behav. 2010 Feb;9(1):33-44

- Duvoisin RM, Villasana L, Pfankuch T, Raber J, Sex-dependent cognitive phenotype of mice lacking mGluR8. Behav Brain Res. 2010 May 1;209(1):21-6

- Robbins MJ, Starr KR, Honey A, Soffin EM, Rourke C, Jones GA, Kelly FM, Strum J, Melarange RA, Harris AJ, Rocheville M, Rupniak T, Murdock PR, Jones DN, Kew JN, Maycox PR, Evaluation of the mGlu8 receptor as a putative therapeutic target in schizophrenia. Brain Res. 2007 Jun 4;1152:215-27

- Duvoisin RM, Zhang C, Pfankuch TF, O'Connor H, Gayet-Primo J, Quraishi S, Raber J, Increased measures of anxiety and weight gain in mice lacking the group III metabotropic glutamate receptor mGluR8. Eur J Neurosci. 2005 Jul;22(2):425-36

- Linden AM, Bergeron M, Baez M, Schoepp DD, Systemic administration of the potent mGlu8 receptor agonist (S)-3,4-DCPG induces c-Fos in stress-related brain regions in wild-type, but not mGlu8 receptor knockout mice. Neuropharmacology. 2003 Sep;45(4):473-83

- Linden AM, Baez M, Bergeron M, Schoepp DD, Increased c-Fos expression in the centromedial nucleus of the thalamus in metabotropic glutamate 8 receptor knockout mice following the elevated plus maze test. Neuroscience. 2003;121(1):167-78

- Zambrowicz BP, Abuin A, Ramirez-Solis R, Richter LJ, Piggott J, BeltrandelRio H, Buxton EC, Edwards J, Finch RA, Friddle CJ, Gupta A, Hansen G, Hu Y, Huang W, Jaing C, Key BW Jr, Kipp P, Kohlhauff B, Ma ZQ, Markesich D, Payne R, Potter DG, Qian N, Shaw J, Schrick J, Shi ZZ, Sparks MJ, Van Sligtenhorst I, Vogel P, Walke W, Xu N, Zhu Q, Person C, Sands AT, Wnk1 kinase deficiency lowers blood pressure in mice: a gene-trap screen to identify potential targets for therapeutic intervention. Proc Natl Acad Sci U S A. 2003 Nov 25;100(24):14109-14

- Zhai J, Tian MT, Wang Y, Yu JL, Koster A, Baez M, Nisenbaum ES, Modulation of lateral perforant path excitatory responses by metabotropic glutamate 8 (mGlu8) receptors. Neuropharmacology. 2002 Aug;43(2):223-30

- Linden AM, Johnson BG, Peters SC, Shannon HE, Tian M, Wang Y, Yu JL, Koster A, Baez M, Schoepp DD, Increased anxiety-related behavior in mice deficient for metabotropic glutamate 8 (mGlu8) receptor. Neuropharmacology. 2002 Aug;43(2):251-9

GPRC2a

Knockout references

http://www.mousephenotype.org/data/genes/MGI:1351351
http://www.informatics.jax.org/marker/MGI:1351351

Phenotype references

- Tang L, Peng M, Liu L, Chang W, Binder HJ, Cheng SX, Calcium-sensing receptor stimulates Cl(-)- and SCFA-dependent but inhibits cAMP-dependent HCO3(-) secretion in colon. Am J Physiol Gastrointest Liver Physiol. 2015 May 15;308(10):G874-83

- Hannan FM, Walls GV, Babinsky VN, Nesbit MA, Kallay E, Hough TA, Fraser WD, Cox RD, Hu J, Spiegel AM, Thakker RV, The Calcilytic Agent NPS 2143 Rectifies Hypocalcemia in a Mouse Model With an Activating Calcium-Sensing Receptor (CaSR) Mutation: Relevance to Autosomal Dominant Hypocalcemia Type 1 (ADH1). Endocrinology. 2015 Sep;156(9):3114-21

- Cheng SX, Lightfoot YL, Yang T, Zadeh M, Tang L, Sahay B, Wang GP, Owen JL, Mohamadzadeh M, Epithelial CaSR deficiency alters intestinal integrity and promotes proinflammatory immune responses. FEBS Lett. 2014 Nov 17;588(22):4158-66

- Quinn SJ, Thomsen AR, Egbuna O, Pang J, Baxi K, Goltzman D, Pollak M, Brown EM, CaSR-mediated interactions between calcium and magnesium homeostasis in mice. Am J Physiol Endocrinol Metab. 2013 Apr 1;304(7):E724-33

- Quinn SJ, Thomsen AR, Pang JL, Kantham L, Brauner-Osborne H, Pollak M, Goltzman D, Brown EM, Interactions between calcium and phosphorus in the regulation of the production of fibroblast growth factor 23 in vivo. Am J Physiol Endocrinol Metab. 2013 Feb 1;304(3):E310-20

- Macleod RJ, Extracellular calcium-sensing receptor/PTH knockout mice colons have increased Wnt/beta-catenin signaling, reduced non-canonical Wnt signaling, and increased susceptibility to azoxymethane- induced aberrant crypt foci. Lab Invest. 2013 May;93(5):520-7

- Mamillapalli R, VanHouten J, Dann P, Bikle D, Chang W, Brown E, Wysolmerski J, Mammary-specific ablation of the calcium-sensing receptor during lactation alters maternal calcium metabolism, mi k calcium transport, and neonatal calcium accrual. Endocrinology. 2013 Sep;154(9):3031-42

- Rey O, Chang W, Bikle D, Rozengurt N, Young SH, Rozengurt E, Negative Cross-talk between Calcium-sensing Receptor and beta-Catenin Signaling Systems in Colonic Epithelium. J Biol Chem. 2012 Jan 6;287(2):1158-67

- Tu CL, Crumrine DA, Man MQ, Chang W, Elalieh H, You M, Elias PM, B kle DD, Ablation of the calcium-sensing receptor in keratinocytes impairs epidermal differentiation and barrier function. J Invest Dermatol. 2012 Oct;132(10):2350-9

- Xue Y, Xiao Y, Liu J, Karaplis AC, Pollak MR, Brown EM, Miao D, Goltzman D, The calcium-sensing receptor complements parathyroid hormone-induced bone turnover in discrete skeletal compartments in mice. Am J Physiol Endocrinol Metab. 2012 Apr;302(7):E841-51

- Sabrautzki S, Rubio-Aliaga I, Hans W, Fuchs H, Rathkolb B, Calzada-Wack J, Cohrs CM, Klaften M, Seedorf H, Eck S, Benet-Pages A, Favor J, Esposito I, Strom TM, Wolf E, Lorenz-Depiereux B, Hrabe de Angelis M, New mouse models for metabolic bone diseases generated by genome-wide ENU mutagenesis. Mamm Genome. 2012 Aug;23(7-8):416-30

- Toka HR, Al-Romaih K, Koshy JM, DiBartolo S 3rd, Kos CH, Quinn SJ, Curhan GC, Mount DB, Brown EM, Pollak MR, Deficiency of the calcium-sensing receptor in the kidney causes parathyroid hormone- independent hypocalciuria. J Am Soc Nephrol. 2012 Nov;23(11):1879-90

- Liu J, Lv F, Sun W, Tao C, Ding G, Karaplis A, Brown E, Goltzman D, Miao D, The Abnormal Phenotypes of Cartilage and Bone in Calcium-Sensing Receptor Deficient Mice Are Dependent on the Actions of Calcium, Phosphorus, and PTH. PLoS Genet. 2011 Sep;7(9):e1002294

- Finney B, Wilkinson WJ, Searchfield L, Cole M, Bailey S, Kemp PJ, Riccardi D, An exon 5-less splice variant of the extracellular calcium-sensing receptor rescues absence of the full-length receptor in the developing mouse lung. Exp Lung Res. 2011 Jun;37(5):269-78

- Shu L, Ji J, Zhu Q, Cao G, Karaplis A, Pollak MR, Brown E, Goltzman D, Miao D, The calcium-sensing receptor mediates bone turnover induced by dietary calcium and parathyroid hormone in neonates. J Bone Miner Res. 2011 May;26(5):1057-71

- Sun W, Liu J, Zhou X, Xiao Y, Karaplis A, Pollak MR, Brown E, Goltzman D, Miao D, Alterations in phosphorus, calcium and PTHrP contr bute to defects in dental and dental alveolar bone formation in calcium- sensing receptor-deficient mice. Development. 2010 Mar;137(6):985-92

- Feng J, Petersen CD, Coy DH, Jiang JK, Thomas CJ, Pollak MR, Wank SA, Calcium-sensing receptor is a physiologic multimodal chemosensor regulating gastric G-cell growth and gastrin secretion. Proc Natl Acad Sci U S A. 2010 Oct 12;107(41):17791-6

- Egbuna O, Quinn S, Kantham L, Butters R, Pang J, Pollak M, Goltzman D, Brown E, The full-length calcium-sensing receptor dampens the calcemic response to 1alpha,25(OH)2 vitamin D3 in vivo independently of parathyroid hormone. Am J Physiol Renal Physiol. 2009 Sep;297(3):F720-8

- Kantham L, Quinn SJ, Egbuna OI, Baxi K, Butters R, Pang JL, Pollak MR, Goltzman D, Brown EM, The calcium-sensing receptor (CaSR) defends against hypercalcemia independently of its regulation of parathyroid hormone secretion. Am J Physiol Endocrinol Metab. 2009 Oct;297(4):E915-23

- Vizard TN, O'Keeffe GW, Gutierrez H, Kos CH, Riccardi D, Davies AM, Regulation of axonal and dendritic growth by the extracellular calcium-sensing receptor. Nat Neurosci. 2008 Mar;11(3):285-91

- Phillips CG, Harnett MT, Chen W, Smith SM, Calcium-sensing receptor activation depresses synaptic transmission. J Neurosci. 2008 Nov 12;28(46):12062-70

- Chang W, Tu C, Chen TH, B kle D, Shoback D, The extracellular calcium-sensing receptor (CaSR) is a critical modulator of skeletal development. Sci Signal. 2008;1(35):ra1

- Chattopadhyay N, Jeong KH, Yano S, Huang S, Pang JL, Ren X, Terwilliger E, Kaiser UB, Vassilev PM, Pollak MR, Brown EM, Calcium receptor stimulates chemotaxis and secretion of MCP-1 in GnRH neurons in vitro: potential impact on reduced GnRH neuron population in CaR-null mice. Am J Physiol Endocrinol Metab. 2007 Feb;292(2):E523-32

GPRC5a

Knockout references

http://www.mousephenotype.org/data/genes/MGI:1891250
http://www.informatics.jax.org/marker/MGI:1891250

Phenotype references

- Zhong S, Yin H, Liao Y, Yao F, Li Q, Zhang J, Jiao H, Zhao Y, Xu D, Liu S, Song H, Gao Y, Liu J, Ma L, Pang Z, Yang R, Ding C, Sun B, Lin X, Ye X, Guo W, Han B, Zhou BP, Chin YE, Deng J, Lung Tumor Suppressor GPRC5A Binds EGFR and Restrains Its Effector Signaling. Cancer Res. 2015 May 1;75(9):1801-14

- Zhang L, Ke F, Liu Z, Bai J, Liu J, Yan S, Xu Z, Lou F, Wang H, Zhu H, Sun Y, Cai W, Gao Y, Li Q, Yu XZ, Qian Y, Hua Z, Deng J, Li QJ, Wang H, MicroRNA-31 negatively regulates peripherally derived regulatory T-cell generation by repressing retinoic acid-inducible protein 3. Nat Commun. 2015;6:7639

- Chen Y, Deng J, Fujimoto J, Kadara H, Men T, Lotan D, Lotan R, Gprc5a deletion enhances the transformed phenotype in normal and malignant lung epithelial cells by eliciting persistent Stat3 signaling induced by autocrine leukemia inhibitory factor. Cancer Res. 2010 Nov 1;70(21):8917-26

- Fujimoto J, Kadara H, Men T, van Pelt C, Lotan D, Lotan R, Comparative functional genomics analysis of NNK tobacco-carcinogen induced lung adenocarcinoma development in Gprc5a-knockout mice. PLoS One. 2010;5(7):e11847

- Hansen GM, Markesich DC, Burnett MB, Zhu Q, Dionne KM, Richter LJ, Finnell RH, Sands AT, Zambrowicz BP, Abuin A, Large-scale gene trapping in C57BL/6N mouse embryonic stem cells. Genome Res. 2008 Oct;18(10):1670-9

- Tao Q, Fujimoto J, Men T, Ye X, Deng J, Lacroix L, Clifford JL, Mao L, Van Pelt CS, Lee JJ, Lotan D, Lotan R, Identification of the retinoic acid-inducible Gprc5a as a new lung tumor suppressor gene. J Natl Cancer Inst. 2007 Nov 21;99(22):1668-82

- Xu J, Tian J, Shapiro SD, Normal Lung Development in RAIG1-Deficient Mice Despite Unique Lung Epithelium-Specific Expression. Am J Respir Cell Mol Biol. 2005 May;32(5):381-7

- Hansen J, Floss T, Van Sloun P, Fuchtbauer EM, Vauti F, Arnold HH, Schnutgen F, Wurst W, von Melchner H, Ruiz P, A large-scale, gene-driven mutagenesis approach for the functional analysis of the mouse genome. Proc Natl Acad Sci U S A. 2003 Aug 19;100(17):9918-22

- Zambrowicz BP, Abuin A, Ramirez-Solis R, Richter LJ, Piggott J, BeltrandelRio H, Buxton EC, Edwards J, Finch RA, Friddle CJ, Gupta A, Hansen G, Hu Y, Huang W, Jaing C, Key BW Jr, Kipp P, Kohlhauff B, Ma ZQ, Markesich D, Payne R, Potter DG, Qian N, Shaw J, Schrick J, Shi ZZ, Sparks MJ, Van Sligtenhorst I, Vogel P, Walke W, Xu N, Zhu Q, Person C, Sands AT, Wnk1 kinase deficiency lowers blood pressure in mice: a gene-trap screen to identify potential targets for therapeutic intervention. Proc Natl Acad Sci U S A. 2003 Nov 25;100(24):14109-14

GPRC5b

Knockout references

http://www.mousephenotype.org/data/genes/MGI:1927596
http://www.informatics.jax.org/marker/MGI:1927596

Phenotype references

- International Mouse Strain Resource, MGI download of germline transmission data for alleles from IMSR strain data. Database Download. 2014;

- Kim YJ, Sano T, Nabetani T, Asano Y, Hirabayashi Y, GPRC5B activates obesity-associated inflammatory signaling in adipocytes. Sci Signal. 2012 Nov 20;5(251):ra85

- Sano T, Kim YJ, Oshima E, Shimizu C, Kiyonari H, Abe T, Higashi H, Yamada K, Hirabayashi Y, Comparative characterization of GPRC5B and GPRC5CLacZ knockin mice; behavioral abnormalities in GPRC5B-deficient mice. Biochem Biophys Res Commun. 2011 Sep 2;412(3):460-5

- Skarnes WC, Rosen B, West AP, Koutsourakis M, Bushell W, Iyer V, Mujica AO, Thomas M, Harrow J, Cox T, Jackson D, Severin J, Biggs P, Fu J, Nefedov M, de Jong PJ, Stewart AF, Bradley A, A conditional knockout resource for the genome-wide study of mouse gene function. Nature. 2011 Jun 16;474(7351):337- 42

- Wellcome Trust Sanger Institute, Alleles produced for the EUCOMM and EUCOMMTools projects by the Wellcome Trust Sanger Institute. MGI Direct Data Submission. 2010;

- Mouse Genome Informatics (MGI) and National Center for Biotechnology Information (NCBI), Mouse Gene Trap Data Load from dbGSS. Database Download. 2008;

- Zambrowicz BP, Abuin A, Ramirez-Solis R, Richter LJ, Piggott J, BeltrandelRio H, Buxton EC, Edwards J, Finch RA, Friddle CJ, Gupta A, Hansen G, Hu Y, Huang W, Jaing C, Key BW Jr, Kipp P, Kohlhauff B, Ma ZQ, Markesich D, Payne R, Potter DG, Qian N, Shaw J, Schrick J, Shi ZZ, Sparks MJ, Van Sligtenhorst I, Vogel P, Walke W, Xu N, Zhu Q, Person C, Sands AT, Wnk1 kinase deficiency lowers blood pressure in mice: a gene-trap screen to identify potential targets for therapeutic intervention. Proc Natl Acad Sci U S A. 2003 Nov 25;100(24):14109-14

8 - Bibliography

1. Katritch V, Cherezov V, Stevens RC. Structure-Function of the G Protein–Coupled Receptor Superfamily. *Annu. Rev. Pharmacol. Toxicol.* 2013;53(1):531–556.
2. Ritter SL, Hall RA. Fine-tuning of GPCR activity by receptor-interacting proteins. *Nature Reviews Molecular Cell Biology*. 2009;10(12):819–830.
3. Gao QB, Wang ZZ. Classification of G-protein coupled receptors at four levels. *Protein Engineering Design and Selection*. 2006;19(11):511–516.
4. Kufareva I, Katritch V, Stevens RC, Abagyan R, 2013 POGD. Advances in GPCR Modeling Evaluated by the GPCR Dock 2013 Assessment: Meeting New Challenges. *Structure/Folding and Design*. 2014;22(8):1120–1139.
5. Attwood TK, Findlay JBC. Design of a discriminating fingerprint for G-protein-coupled receptors. *Protein Engineering Design and Selection*. 1993;6(2):167–176.
6. Kolakowski LF. GCRDb: a G-protein-coupled receptor database. *Recept. Channels*. 1994;2(1):1–7.
7. Bockaert J, Pin JP. Molecular tinkering of G protein-coupled receptors: an evolutionary success. *EMBO J.* 1999;18(7):1723–1729.
8. Richards JE, Hawley RS. The Human Genome Sequence. *The Human Genome*. 2005;299–310.
9. Fredriksson R. The G-Protein-Coupled Receptors in the Human Genome Form Five Main Families. Phylogenetic Analysis, Paralogon Groups, and Fingerprints. *Molecular Pharmacology*. 2003;63(6):1256–1272.
10. Katritch V, Cherezov V, Stevens RC. Diversity and modularity of G protein-coupled receptor structures. *Trends in Pharmacological Sciences*. 2012;33(1):17–27.
11. Lagerström MC, Schiöth HB. Structural diversity of G protein-coupled receptors and significance for drug discovery. *Nature Reviews Drug Discovery*. 2008;7(4):339–357.
12. Cardoso JCR, Félix RC, Fonseca VG, Power DM. Feeding and the Rhodopsin Family G-Protein Coupled Receptors in Nematodes and Arthropods. *Frontiers in Endocrinology*. 2012;3:157.
13. Gupte J, Swaminath G, Danao J, et al. Signaling property study of adhesion G-protein-coupled receptors. *FEBS Letters*. 2012;586(8):1214–1219.
14. Yona S, Lin H-H, Siu WO, Gordon S, Stacey M. Adhesion-GPCRs: emerging roles for novel receptors. *Trends in Biochemical Sciences*. 2008;33(10):491–500.
15. Yona S, Lin H-H, Stacey M. Immunity and adhesion-GPCRs. *Adv. Exp. Med. Biol.* 2010;706:121–127.
16. Strotmann R, Schröck K, Böselt I, et al. Evolution of GPCR: change and continuity. *Mol. Cell. Endocrinol.* 2011;331(2):170–178.
17. Schöneberg T, Hofreiter M, Schulz A, Römpler H. Learning from the past: evolution of GPCR functions. *Trends in Pharmacological Sciences*. 2007;28(3):117–121.
18. Oldham WM, Hamm HE. Heterotrimeric G protein activation by G-protein-coupled receptors. *Nature Reviews Molecular Cell Biology*. 2008;9(1):60–71.
19. Chidiac P. RGS proteins destroy spare receptors: Effects of GPCR-interacting proteins and signal deamplification on measurements of GPCR agonist potency. *Methods*. 2016;92:87–93.
20. Dohlman HG. Regulators of G Protein Signaling (RGS Proteins). Hoboken, NJ, USA: John Wiley & Sons, Inc; 2002.
21. Rasmussen SGF, DeVree BT, Zou Y, et al. Crystal structure of the β2 adrenergic receptor-Gs protein complex. *Nature*. 2011;477(7366):549–555.
22. Berstein G, Blank JL, Jhon D-Y, et al. Phospholipase C-β1 is a GTPase-activating protein for Gq/11, its physiologic regulator. *Cell*. 1992;70(3):411–418.
23. Brown HA, Gutowski S, Moomaw CR, Slaughter C, Sternwels PC. ADP-ribosylation factor, a small GTP-dependent regulatory protein, stimulates phospholipase D activity. *Cell*. 1993;75(6):1137–1144.

24. Popova JS, Johnson GL, Rasenick MM. Chimeric G alpha s/G alpha i2 proteins define domains on G alpha s that interact with tubulin for beta-adrenergic activation of adenylyl cyclase. *Journal of Biological Chemistry*. 1994;269(34):21748–21754.
25. Roychowdhury S, Panda D, Wilson L, Rasenick MM. G protein alpha subunits activate tubulin GTPase and modulate microtubule polymerization dynamics. *Journal of Biological Chemistry*. 1999;274(19):13485–13490.
26. Parsons SJ, Parsons JT. Src family kinases, key regulators of signal transduction. *Oncogene*. 2004;23(48):7906–7909.
27. Ahnert-Hilger G, Schäfer T, Spicher K, et al. Detection of G-protein heterotrimers on large dense core and small synaptic vesicles of neuroendocrine and neuronal cells. *Eur J Cell Biol*. 1994;65(1):26–38.
28. Oleskevich S. G alpha o1 decapeptide modulates the hippocampal 5-HT1A potassium current. *Journal of Neurophysiology*. 1995;74(5):2189–2193.
29. Kehrl JH. G-Protein-Coupled Receptor Signaling, RGS Proteins, and Lymphocyte Function. *CRI*. 2004;24(6):16.
30. Boularan C, Kehrl JH. Implications of non-canonical G-protein signaling for the immune system. *Cell. Signal.* 2014;26(6):1269–1282.
31. Jeong S-W, Ikeda SR. G Protein α Subunit Gαz Couples Neurotransmitter Receptors to Ion Channels in Sympathetic Neurons. *Neuron*. 1998;21(5):1201–1212.
32. Morris TA, Fong SL. Characterization of the gene encoding human cone transducin α-subunit (GNAT2). *Genomics*. 1993.
33. Hao W, Wenzel A, Obin MS, et al. Evidence for two apoptotic pathways in light-induced retinal degeneration. *Nature Genetics*. 2002;32(2):254–260.
34. Kusakabe Y, Yasuoka A, Asano-Miyoshi M, et al. Comprehensive study on G protein alpha-subunits in taste bud cells, with special reference to the occurrence of Galphai2 as a major Galpha species. *Chem. Senses*. 2000;25(5):525–531.
35. Neves SR, Ram PT, Iyengar R. G Protein Pathways. *Science*. 2002;296(5573):1636–1639.
36. Kostenis E, Waelbroeck M, Milligan G. Techniques: promiscuous Galpha proteins in basic research and drug discovery. *Trends in Pharmacological Sciences*. 2005;26(11):595–602.
37. Hermans E. Biochemical and pharmacological control of the multiplicity of coupling at G-protein-coupled receptors. *Pharmacology and Therapeutics*. 2003;99(1):25–44.
38. Kostenis E. Is Gα16 the optimal tool for fishing ligands of orphan G-protein-coupled receptors? *Trends in Pharmacological Sciences*. 2001;22(11):560–564.
39. Liu B, Wu D. Analysis of the Coupling of G_{12/13} to G Protein-Coupled Receptors Using a Luciferase Reporter Assay. *G Protein Signaling*. 2003;237:145–150.
40. Strathmann MP, Simon MI. G alpha 12 and G alpha 13 subunits define a fourth class of G protein alpha subunits. *Proceedings of the National Academy of Sciences*. 1991;88(13):5582–5586.
41. Kruse AC, Ring AM, Manglik A, et al. Structure of active human M2 muscarinic acetylcholine receptor bound to the agonist iperoxo. 2013.
42. Kruse AC, Ring AM, Manglik A, et al. Activation and allosteric modulation of a muscarinic acetylcholine receptor. *Nature*. 2013;504(7478):101–106.
43. Kruse AC, Hu J, Pan AC, et al. Structure and dynamics of the M3 muscarinic acetylcholine receptor. *Nature*. 2012;482(7386):552–556.
44. Thorsen TS, Matt R, Weis WI, Kobilka BK. Modified T4 Lysozyme Fusion Proteins Facilitate G Protein-Coupled Receptor Crystallogenesis. *Structure*. 2014;22(11):1657–1664.
45. Chien EYT, Liu W, Zhao Q, et al. Structure of the human dopamine D3 receptor in complex with a D2/D3 selective antagonist. *Science*. 2010;330(6007):1091–1095.
46. Wang C, Jiang Y, Ma J, et al. Structural basis for molecular recognition at serotonin receptors. *Science*. 2013;340(6132):610–614.
47. Wacker D, Wang C, Katritch V, et al. Structural Features for Functional Selectivity at Serotonin Receptors. *Science*. 2013;340(6132):615–619.

48. Liu W, Wacker D, Gati C, et al. Serial femtosecond crystallography of G protein-coupled receptors. *Science*. 2013;342(6165):1521–1524.
49. Huang J, Chen S, Zhang JJ, Huang X-Y. Crystal structure of oligomeric β1-adrenergic G protein–coupled receptors in ligand-free basal state. *Nature Structural & Molecular Biology*. 2013;20(4):419–425.
50. Palczewski K, Kumasaka T, Hori T, et al. Crystal structure of rhodopsin: A G protein-coupled receptor. *Science*. 2000;289(5480):739–745.
51. Shimamura T, Shiroishi M, Weyand S, et al. Structure of the human histamine H1 receptor complex with doxepin. *Nature*. 2011;475(7354):65–70.
52. Jaakola V-P, Griffith MT, Hanson MA, et al. The 2.6 angstrom crystal structure of a human A2A adenosine receptor bound to an antagonist. *Science*. 2008;322(5905):1211–1217.
53. Ashok Y, Nanekar RT, Jaakola V-P. Crystallogenesis of Adenosine A2A Receptor—T4 Lysozyme Fusion Protein. *G Protein Coupled Receptors - Structure*. 2013;520:175–198.
54. Zhang J, Zhang K, Gao Z-G, et al. Agonist-bound structure of the human P2Y12 receptor. *Nature*. 2014;509(7498):119–122.
55. Zhang K, Zhang J, Gao Z-G, et al. Structure of the human P2Y12 receptor in complex with an antithrombotic drug. *Nature*. 2014;509(7498):115–118.
56. Zhang C, Srinivasan Y, Arlow DH, et al. High-resolution crystal structure of human protease-activated receptor 1. *Nature*. 2012;492(7429):387–392.
57. White JF, Noinaj N, Shibata Y, et al. Structure of the agonist-bound neurotensin receptor. …. 2012.
58. Hollenstein K, Kean J, Bortolato A, et al. Structure of class B GPCR corticotropin-releasing factor receptor 1. *Nature*. 2013;499(7459):438–443.
59. Siu FY, He M, de Graaf C, et al. Structure of the human glucagon class B G-protein-coupled receptor. *Nature*. 2013;499(7459):444–449.
60. Wang C, Wu H, Evron T, et al. Structural basis for Smoothened receptor modulation and chemoresistance to anticancer drugs. *Nature Communications*. 2014;5:.
61. Wu H, Wang C, Gregory KJ, et al. Structure of a class C GPCR metabotropic glutamate receptor 1 bound to an allosteric modulator. *Science*. 2014;344(6179):58–64.
62. Wu B, Chien EYT, Mol CD, et al. Structures of the CXCR4 chemokine GPCR with small-molecule and cyclic peptide antagonists. *Science*. 2010;330(6007):1066–1071.
63. Tan Q, Zhu Y, Li J, et al. Structure of the CCR5 chemokine receptor-HIV entry inhibitor maraviroc complex. *Science*. 2013;341(6152):1387–1390.
64. Thompson AA, Liu W, Chun E, et al. Structure of the nociceptin/orphanin FQ receptor in complex with a peptide mimetic. *Nature*. 2012;485(7398):395–399.
65. Wu H, Wacker D, Mileni M, et al. Structure of the human [kgr]-opioid receptor in complex with JDTic. *Nature*. 2012;485(7398):327–332.
66. Manglik A, Kruse AC, Kobilka TS, et al. Crystal structure of the [micro]-opioid receptor bound to a morphinan antagonist. *Nature*. 2012;485(7398):321–326.
67. Granier S, Manglik A, Kruse AC, et al. Structure of the δ-opioid receptor bound to naltrindole. *Nature*. 2012;485(7398):400–404.
68. Scheerer P, Park JH, Hildebrand PW, et al. Crystal structure of opsin in its G-protein-interacting conformation. *Nature*. 2008;455(7212):497–502.
69. Yin X, Xu H, Hanson M, Liu W. GPCR Crystallization Using Lipidic Cubic Phase Technique. 2014.
70. Bertheleme N, Chae PS, Singh S, et al. Unlocking the secrets of the gatekeeper: Methods for stabilizing and crystallizing GPCRs. *Biochimica et Biophysica Acta (BBA) - Biomembranes*. 2013;1828(11):2583–2591.
71. Choe H-W, Park JH, Kim YJ, Ernst OP. Transmembrane signaling by GPCRs: Insight from rhodopsin and opsin structures. *Neuropharmacology*. 2011;60(1):52–57.
72. Valentin-Hansen L, Groenen M, Nygaard R, et al. The arginine of the DRY motif in transmembrane segment III functions as a balancing micro-switch in the activation of the β2-adrenergic receptor. *J. Biol. Chem.* 2012;287(38):31973–31982.

73. Nygaard R, Zou Y, Dror RO, et al. The dynamic process of β(2)-adrenergic receptor activation. *Cell*. 2013;152(3):532–542.
74. Standfuss J, Xie G, Edwards PC, et al. Crystal Structure of a Thermally Stable Rhodopsin Mutant. *Journal of Molecular Biology*. 2007;372(5):1179–1188.
75. Rasmussen SGF, Gether U. Molecular Mechanisms of GPCR Activation. *G Protein-Coupled Receptors as Drug Targets*. 2006;27–42.
76. Bond RA, IJzerman AP. Recent developments in constitutive receptor activity and inverse agonism, and their potential for GPCR drug discovery. *Trends in Pharmacological Sciences*. 2006;27(2):92–96.
77. Strange PG. Three-state and two-state models. *Trends in Pharmacological Sciences*. 1998;19(3):85–86.
78. Lefkowitz RJ, Stadel JM. Adenylate cyclase-coupled beta-adrenergic receptors: structure and mechanisms of activation and desensitization. *Annual review of ….* 1983.
79. Stiles GL, Caron MG, Lefkowitz RJ. Beta-adrenergic receptors: biochemical mechanisms of physiological regulation. *Physiological Reviews*. 1984;64(2):661–743.
80. Kristiansen K. Molecular mechanisms of ligand binding, signaling, and regulation within the superfamily of G-protein-coupled receptors: molecular modeling and mutagenesis approaches to receptor structure and function. *Pharmacology and Therapeutics*. 2004;103(1):21–80.
81. Ahuja S, Smith SO. Multiple Switches in G Protein-Coupled Receptor Activation. *Trends in Pharmacological Sciences*. 2009;30(9):494–502.
82. Jensen PC, Rosenkilde MM. Chapter 8 Activation Mechanisms of Chemokine Receptors. *Chemokines, Part B*. 2009;461:171–190.
83. Saito T, Bunnett NW. Protease-activated receptors. *Neuromol Med*. 2005;7(1-2):79–99.
84. Vergnolle N, Wallace JL, Bunnett NW, Hollenberg MD. Protease-activated receptors in inflammation, neuronal signaling and pain. *Trends in Pharmacological Sciences*. 2001;22(3):146–152.
85. Coughlin SR. Protease-activated receptors in hemostasis, thrombosis and vascular biology. *Journal of Thrombosis and Haemostasis*. 2005;3(8):1800–1814.
86. Pin JP, Kniazeff J, Goudet C, et al. The activation mechanism of class-C G-protein coupled receptors. *Biology of the Cell*. 2004;96(5):335–342.
87. Peeters MC, van Westen GJP, Li Q, IJzerman AP. Importance of the extracellular loops in G protein-coupled receptors for ligand recognition and receptor activation. *Trends in Pharmacological Sciences*. 2011;32(1):35–42.
88. Park PSH, Lodowski DT, Palczewski K. Activation of G Protein–Coupled Receptors: Beyond Two-State Models and Tertiary Conformational Changes. *Annu. Rev. Pharmacol. Toxicol.* 2008;48(1):107–141.
89. Hofmann KP, Jäger S, Ernst OP. Structure and Function of Activated Rhodopsin. *Israel Journal of Chemistry*. 1995;35(3-4):339–355.
90. Jacobsen RB, Sale KL, Ayson MJ, Novak P. Structure and dynamics of dark-state bovine rhodopsin revealed by chemical cross-linking and high-resolution mass spectrometry. *Protein ….* 2006.
91. Rankovic Z, Brust TF, Bohn LM. Biased agonism: An emerging paradigm in GPCR drug discovery. *Bioorg. Med. Chem. Lett.* 2016;26(2):241–250.
92. Violin JD, Crombie AL, Soergel DG, Lark MW. Biased ligands at G-protein-coupled receptors: promise and progress. *Trends in Pharmacological Sciences*. 2014;35(7):308–316.
93. Kelly E, Bailey CP, Henderson G. Agonist-selective mechanisms of GPCR desensitization. *Br J Pharmacol*. 2008;153(S1):S379–S388.
94. Gether U, Kobilka BK. G protein-coupled receptors. II. Mechanism of agonist activation. *Journal of Biological Chemistry*. 1998;273(29):17979–17982.
95. Kroeze WK, Sassano MF, Huang X-P, et al. PRESTO-Tango as an open-source resource for interrogation of the druggable human GPCRome. *Nature Structural & Molecular Biology*. 2015;22(5):362–369.

96. Watanabe Y, Tsujimura A, Aoki M, Taguchi K, Tanaka M. Development of the 5-HT2CR-Tango System Combined with an EGFP Reporter Gene. *J. Mol. Neurosci.* 2015;1–8.
97. Zhang H, Unal H, Desnoyer R, et al. Structural Basis for Ligand Recognition and Functional Selectivity at Angiotensin Receptor. *J. Biol. Chem.* 2015;290(49):29127–29139.
98. Gaven F, Pellissier LP, Queffeulou E, et al. Pharmacological profile of engineered 5-HT_4 receptors and identification of 5-HT_4 receptor-biased ligands. *Brain Research*. 2013;1511:65–72.
99. Manfra O, Van Craenenbroeck K, Skieterska K, et al. Downregulation of 5-HT7 Serotonin Receptors by the Atypical Antipsychotics Clozapine and Olanzapine. Role of Motifs in the C-Terminal Domain and Interaction with GASP-1. *ACS Chem. Neurosci.* 2015;6(7):1206–1218.
100. Kamal FA, Travers JG, Blaxall BC. Embracing bias: β1-adrenergic receptor-biased ligands and nuclear miRNA processing. *Circ. Res.* 2014;114(5):742–745.
101. Cottingham C, Ferryman CJ, Wang Q. α2 Adrenergic Receptor Trafficking as a Therapeutic Target in Antidepressant Drug Action. *Prog Mol Biol Transl Sci*. 2015;132:207–225.
102. Allen JA, Yost JM, Setola V, et al. Discovery of β-arrestin-biased dopamine D2 ligands for probing signal transduction pathways essential for antipsychotic efficacy. *Proc. Natl. Acad. Sci. U.S.A.* 2011;108(45):18488–18493.
103. Wang L-D, Gantz I, Butler K, Hoeltzel M, Del Valle J. Histamine H2 Receptor Mediated Dual Signaling: Mapping of Structural Requirements Using β2 Adrenergic Chimeric Receptors. *Biochemical and Biophysical Research Communications*. 2000;276(2):539–545.
104. Alonso N, Monczor F, Echeverría E, et al. Signal transduction mechanism of biased ligands at histamine H2 receptors. *Biochem. J.* 2014;459(1):117–126.
105. Nijmeijer S, Vischer HF, Sirci F, et al. Detailed analysis of biased histamine H_4 receptor signalling by JNJ 7777120 analogues. *Br J Pharmacol*. 2013;170(1):78–88.
106. van Der Lee MMC, Bras M, van Koppen CJ, Zaman GJR. beta-Arrestin recruitment assay for the identification of agonists of the sphingosine 1-phosphate receptor EDG1. *J Biomol Screen*. 2008;13(10):986–998.
107. Jorgensen R, Kubale V, Vrecl M, Schwartz TW, Elling CE. Oxyntomodulin differentially affects glucagon-like peptide-1 receptor beta-arrestin recruitment and signaling through Galpha(s). *J. Pharmacol. Exp. Ther.* 2007;322(1):148–154.
108. Chung S, Funakoshi T, Civelli O. Orphan GPCR research. *British journal of …*.2008.
109. Civelli O, Saito Y, Wang Z, Nothacker HP. Orphan GPCRs and their ligands. *Pharmacology & …*. 2006.
110. Civelli O, Reinscheid RK, Zhang Y, et al. G Protein–Coupled Receptor Deorphanizations. *Annu. Rev. Pharmacol. Toxicol.* 2013;53(1):127–146.
111. Lecca D, Abbracchio MR. Deorphanisation of G protein-coupled receptors: A tool to provide new insights in nervous system pathophysiology and new targets for psycho-active drugs. *Neurochemistry International*. 2008;52(3):339–351.
112. LEVOYE A. Alternative drug discovery approaches for orphan GPCRs. *Drug Discovery Today*. 2008;13(1-2):52–58.
113. Civelli O, Reinscheid RK, Zhang Y, et al. G Protein–Coupled Receptor Deorphanizations. *Annu. Rev. Pharmacol. Toxicol.* 2013;53(1):127–146.
114. Ahmad R, Wojciech S, Jockers R. Hunting for the function of orphan GPCRs -beyond the search for the endogenous ligand. *Br J Pharmacol*. 2015;172(13):3212–3228.
115. Foord SM, Bonner TI, Neubig RR, et al. International Union of Pharmacology. XLVI. G protein-coupled receptor list. *Pharmacological Reviews*. 2005;57(2):279–288.
116. Insel PA, Snead A, Murray F, et al. GPCR expression in tissues and cells: Are the optimal receptors being used as drug targets? *Br J Pharmacol*. 2012;165(6):1613–1616.
117. Regard JB, Sato IT, Coughlin SR. Anatomical Profiling of G Protein-Coupled Receptor Expression. *Cell*. 2008;135(3):561–571.

118. Alexander S, Benson HE. The Concise Guide to PHARMACOLOGY 2013/14: G Protein-Coupled Receptors. *British journal of* …. 2013.
119. Towner RA, Jensen RL, Colman H, et al. ELTD1, A Potential New Biomarker for Gliomas. *Neurosurgery*. 2013;72(1):77–91.
120. McKnight AJ, Gordon S. The EGF-TM7 family: unusual structures at the leukocyte surface. *J Leukoc Biol*. 1998;63(3):271–280.
121. Li S, Huang S, Peng S-B. Overexpression of G protein-coupled receptors in cancer cells: Involvement in tumor progression. *International Journal of Oncology*. 2005;27(5):1329–1338.
122. Feigin ME, Xue B, Hammell MC, Muthuswamy SK. G-protein-coupled receptor GPR161 is overexpressed in breast cancer and is a promoter of cell proliferation and invasion. *Proceedings of the National Academy of Sciences*. 2014;111(11):4191–4196.
123. Foulkes WD, Smith IE, Reis-Filho JS. Triple-Negative Breast Cancer. *N Engl J Med*. 2010;363(20):1938–1948.
124. Singh A, Nunes JJ, Ateeq B. Role and therapeutic potential of G-protein coupled receptors in breast cancer progression and metastases. *European Journal of Pharmacology*. 2015;763:178–183.
125. Montpetit A, Sinnett D. Physical mapping of the G-protein coupled receptor 19 (GPR19) in the chromosome 12p12.3 region frequently rearranged in cancer cells. *Hum Genet*. 1999;105(1-2):162–164.
126. Kastner S, Voss T, Keuerleber S, et al. Expression of G protein-coupled receptor 19 in human lung cancer cells is triggered by entry into S-phase and supports G(2)-M cell-cycle progression. *Mol Cancer Res*. 2012;10(10):1343–1358.
127. Sokolenko AP, Bulanova DR, Iyevleva AG, et al. High prevalence of GPRC5A germline mutations in BRCA1-mutant breast cancer patients. *Int. J. Cancer*. 2014;134(10):2352–2358.
128. Zhou H, Rigoutsos I. The emerging roles of GPRC5A in diseases. *Oncoscience*. 2014;1(12):765–776.
129. Baens M, Finalet Ferreiro J, Tousseyn T, et al. t(X;14)(p11.4;q32.33) is recurrent in marginal zone lymphoma and up-regulates GPR34. *Haematologica*. 2012;97(2):184–188.
130. Ansell SM, Akasaka T, McPhail E, et al. t(X;14)(p11;q32) in MALT lymphoma involving GPR34 reveals a role for GPR34 in tumor cell growth. *Blood*. 2012;120(19):3949– 3957.
131. Meyer RC, Giddens MM, Schaefer SA, Hall RA. GPR37 and GPR37L1 are receptors for the neuroprotective and glioprotective factors prosaptide and prosaposin. *Proc. Natl. Acad. Sci. U.S.A.* 2013;110(23):9529–9534.
132. Lopes JP, Morató X, Souza C, et al. The role of parkinson's disease-associated receptor GPR37 in the hippocampus: functional interplay with the adenosinergic system. *J. Neurochem.* 2015;134(1):135–146.
133. Benoit ME, Hernandez MX, Dinh ML, et al. C1q-induced LRP1B and GPR6 proteins expressed early in Alzheimer disease mouse models, are essential for the C1q-mediated protection against amyloid-β neurotoxicity. *J. Biol. Chem.* 2013;288(1):654–665.
134. Padmanabhan S, Myers AG, Prasad BM. Constitutively active GPR6 is located in the intracellular compartments. *FEBS Letters*. 2009;583(1):107–112.
135. Komatsu H, Maruyama M, Yao S, et al. Anatomical transcriptome of G protein-coupled receptors leads to the identification of a novel therapeutic candidate GPR52 for psychiatric disorders. *PLoS ONE*. 2014;9(2):e90134.
136. Sawzdargo M, Nguyen T, Lee DK, et al. Identification and cloning of three novel human G protein-coupled receptor genes GPR52, PsiGPR53 and GPR55: GPR55 is extensively expressed in human brain. *Brain Res. Mol. Brain Res.* 1999;64(2):193–198.
137. Ingallinesi M, Le Bouil L, Biguet NF, et al. Local inactivation of Gpr88 in the nucleus accumbens attenuates behavioral deficits elicited by the neonatal administration of

phencyclidine in rats. *Molecular Psychiatry*. 2015;20(8):951–958.
138. Meirsman AC, Le Merrer J, Pellissier LP, et al. Mice Lacking GPR88 Show Motor Deficit, Improved Spatial Learning, and Low Anxiety Reversed by Delta Opioid Antagonist. *Biol. Psychiatry*. 2015.
139. Bi Y, Dzierba CD, Fink C, et al. The discovery of potent agonists for GPR88, an orphan GPCR, for the potential treatment of CNS disorders. *Bioorg. Med. Chem. Lett.* 2015;25(7):1443–1447.
140. Gardner J, Wu S, Ling L, et al. G-protein-coupled receptor GPR21 knockout mice display improved glucose tolerance and increased insulin response. *Biochemical and Biophysical Research Communications*. 2012;418(1):1–5.
141. Resnick HE. Deletion of Gpr21 protects against obesity-associated inflammation and insulin resistance. Diabetes; 2012.
142. Chen D, Liu X, Zhang W, Shi Y. Targeted Inactivation of GPR26 Leads to Hyperphagia and Adiposity by Activating AMPK in the Hypothalamus. *PLoS ONE*. 2012;7(7):e40764.
143. Hendrick A, Dixon J, Tait TM, Fradley R. GPR 26 G-Protein Coupled Receptor. 2006;(12/ …):.
144. Ku GM, Pappalardo Z, Luo CC, German MS, McManus MT. An siRNA Screen in Pancreatic Beta Cells Reveals a Role for Gpr27 in Insulin Production. *PLOS Genet*. 2012;8(1):e1002449.
145. H AR, Hong E, Sanchez-Muñoz F, Villafaña S. Expression of the orphan receptors GPR22, GPR162, GPR27 and GPR17 in cardiovascular tissues of diabetic rats (1051.7). *The FASEB Journal*. 2014;28(1 Supplement):1051.7.
146. Ivanova EA, Bechtold DA, Dupré SM, et al. Altered metabolism in the melatonin-related receptor (GPR50) knockout mouse. *American Journal of Physiology - Endocrinology and Metabolism*. 2008;294(1):E176–E182.
147. Bhattacharyya S, Luan J, Challis B, et al. Sequence variants in the melatonin-related receptor gene (GPR50) associate with circulating triglyceride and HDL levels. *The Journal of Lipid Research*. 2006;47(4):761–766.
148. Engel KMY, Schröck K, Teupser D, et al. Reduced Food Intake and Body Weight in Mice Deficient for the G Protein-Coupled Receptor GPR82. *PLoS ONE*. 2011;6(12):e29400.
149. Engel K. The Physiological Relevance of the Orphan G Protein-coupled Receptor GPR82. 2011.
150. Levoye A, Dam J, Ayoub MA, Guillaume J-L, Jockers R. Do orphan G-protein-coupled receptors have ligand-independent functions? New insights from receptor heterodimers. *EMBO Rep*. 2006;7(11):1094–1098.
151. Ahmad R, Wojciech S, Jockers R. Hunting for the function of orphan GPCRs -beyond the search for the endogenous ligand. *Br J Pharmacol*. 2014;172(13):3212–3228.
152. Martin AL, Steurer MA, Aronstam RS. Constitutive Activity among Orphan Class-A G Protein Coupled Receptors. *PLoS ONE*. 2015;10(9):e0138463.
153. Freudzon L, Norris RP, Hand AR, et al. Regulation of meiotic prophase arrest in mouse oocytes by GPR3, a constitutive activator of the Gs G protein. *J. Cell Biol.* 2005;171(2):255–265.
154. Martin AL, Steurer MA, Aronstam RS. Constitutive Activity among Orphan Class-A G Protein Coupled Receptors. *PLoS ONE*. 2015;10(9):e0138463.
155. Toyooka M, Tujii T, Takeda S. The N-terminal domain of GPR61, an orphan G-protein-coupled receptor, is essential for its constitutive activity. *J. Neurosci. Res.* 2009;87(6):1329–1333.
156. Sugita K, Yamamura C, Tabata K-I, Fujita N. Expression of orphan G-protein coupled receptor GPR174 in CHO cells induced morphological changes and proliferation delay via increasing intracellular cAMP. *Biochemical and Biophysical Research Communications*. 2013;430(1):190–195.
157. Napier C, Mitchell AL, Gan E, Wilson I, Pearce SHS. Role of the X-linked gene GPR174 in autoimmune Addison's disease. *J. Clin. Endocrinol. Metab.* 2015;100(1):E187–90.

158. Barnes MJ, Li C-M, Xu Y, et al. The lysophosphatidylserine receptor GPR174 constrains regulatory T cell development and function. *J. Exp. Med.* 2015;212(7):1011–1020.
159. Kristinsson H, Bergsten P, Sargsyan E. Free fatty acid receptor 1 (FFAR1/GPR40) signaling affects insulin secretion by enhancing mitochondrial respiration during palmitate exposure. *Biochim. Biophys. Acta*. 2015;1853(12):3248–3257.
160. Laffray S, Tan K, Dulluc J, et al. Dissociation and trafficking of rat GABAB receptor heterodimer upon chronic capsaicin stimulation. *Eur. J. Neurosci.* 2007;25(5):1402–1416.
161. Laffray S, Bouali-Benazzouz R, Papon M-A, et al. Impairment of GABAB receptor dimer by endogenous 14-3-3ζ in chronic pain conditions. *EMBO J.* 2012;31(15):3239– 3251.
162. Ray TA, Heath KM, Hasan N, et al. GPR179 is required for high sensitivity of the mGluR6 signaling cascade in depolarizing bipolar cells. *J. Neurosci.* 2014;34(18):6334–6343.
163. Nishiguchi KM, Carvalho LS, Rizzi M, et al. Gene therapy restores vision in rd1 mice after removal of a confounding mutation in Gpr179. *Nature Communications*. 2015;6:6006.
164. Levoye A, Dam J, Ayoub MA, et al. The orphan GPR50 receptor specifically inhibits MT1 melatonin receptor function through heterodimerization. *EMBO J.* 2006;25(13):3012–3023.
165. Jockers R, Maurice P, Boutin JA, Delagrange P. Melatonin receptors, heterodimerization, signal transduction and binding sites: what's new? *Br J Pharmacol*. 2008;154(6):1182–1195.
166. Milasta S, Pediani J, Appelbe S, Trim S, Wyatt M. Interactions between the Mas- related receptors MrgD and MrgE alter signalling and trafficking of MrgD. *Molecular* …. 2006.
167. Marazziti D, Mandillo S, Di Pietro C, et al. GPR37 associates with the dopamine transporter to modulate dopamine uptake and behavioral responses to dopaminergic drugs. *Proceedings of the National Academy of Sciences*. 2007;104(23):9846–9851.
168. Marazziti D, Di Pietro C, Mandillo S, et al. Absence of the GPR37/PAEL receptor impairs striatal Akt and ERK2 phosphorylation, DeltaFosB expression, and conditioned place preference to amphetamine and cocaine. *FASEB J.* 2011;25(6):2071–2081.
169. Müllner C, Vorobiov D, Bera AK, et al. Heterologous facilitation of G protein-activated K(+) channels by beta-adrenergic stimulation via cAMP-dependent protein kinase. *J Gen Physiol*. 2000;115(5):547–558.
170. Duane D Hall, Monika A Davare, Mei Shi, et al. Critical Role of cAMP-Dependent Protein Kinase Anchoring to the L-Type Calcium Channel Cav1.2 via A-Kinase Anchor Protein 150 in Neurons†. *Biochemistry*. 2007;46(6):1635–1646.
171. Alvarez-Curto E, Prihandoko R, Tautermann CS, et al. Developing chemical genetic approaches to explore G protein-coupled receptor function: validation of the use of a receptor activated solely by synthetic ligand (RASSL). *Molecular Pharmacology*. 2011;80(6):1033–1046.
172. Wess J, Nakajima K, Jain S. Novel designer receptors to probe GPCR signaling and physiology. *Trends in Pharmacological Sciences*. 2013;34(7):385–392.
173. Bruysters M, Jongejan A, Akdemir A, Bakker RA, Leurs R. A G(q/11)-coupled mutant histamine H(1) receptor F435A activated solely by synthetic ligands (RASSL). *Journal of Biological Chemistry*. 2005;280(41):34741–34746.
174. Sweger EJ, Casper KB, Scearce-Levie K, Conklin BR, McCarthy KD. Development of hydrocephalus in mice expressing the G(i)-coupled GPCR Ro1 RASSL receptor in astrocytes. *J. Neurosci.* 2007;27(9):2309–2317.
175. Zhao GQ, Zhang Y, Hoon MA, et al. The Receptors for Mammalian Sweet and Umami Taste. *Cell*. 2003;115(3):255–266.
176. Conklin BR, Hsiao EC, Claeysen S, et al. Engineering GPCR signaling pathways with RASSLs. *Nat Meth*. 2008;5(8):673–678.
177. Zhu H, Roth BL. DREADD: a chemogenetic GPCR signaling platform. *Int. J.*

Neuropsychopharmacol. 2015;18(1):pyu007–pyu007.
178. Farrell MS, Roth BL. Pharmacosynthetics: Reimagining the pharmacogenetic approach. *Brain Research*. 2013;1511:6–20.
179. Yin D. Probing Receptor Structure/Function with Chimeric G-Protein-Coupled Receptors. *Molecular Pharmacology*. 2004;65(6):1323–1332.
180. Li H, Malbon CC, Wang H-Y. Gene profiling of Frizzled-1 and Frizzled-2 signaling: expression of G-protein-coupled receptor chimeras in mouse F9 teratocarcinoma embryonal cells. *Molecular Pharmacology*. 2004;65(1):45–55.
181. DeCostanzo AJ, Huang X-P, Wang H-Y, Malbon CC. The Frizzled-1/(beta(2))-adrenergic receptor chimera: pharmacological properties of a unique G protein-linked receptor. *Naunyn-Schmiedeberg's Archives of Pharmacology*. 2002;365(5):341–348.
182. Kozell LB, Neve KA. Constitutive activity of a chimeric D2/D1 dopamine receptor. *Molecular Pharmacology*. 1997;52(6):1137–1149.
183. Stujenske JM, Spellman T, Gordon JA. Modeling the Spatiotemporal Dynamics of Light and Heat Propagation for In Vivo Optogenetics. *Cell Rep*. 2015;12(3):525–534.
184. Long X, Ye J, Zhao D, Zhang S-J. Magnetogenetics: remote non-invasive magnetic activation of neuronal activity with a magnetoreceptor. *Sci Bull (Beijing)*. 2015;60(24):2107–2119.
185. Etoc F, Vicario C, Lisse D, et al. Magnetogenetic control of protein gradients inside living cells with high spatial and temporal resolution. *Nano Lett.* 2015;15(5):3487–3494.
186. Etoc F, Lisse D, Bellaiche Y, et al. Subcellular control of Rac-GTPase signalling by magnetogenetic manipulation inside living cells. *Nat Nanotechnol*. 2013;8(3):193–198.
187. Bonnemay L, Hostachy S, Hoffmann C, Gautier J, Gueroui Z. Engineering spatial gradients of signaling proteins using magnetic nanoparticles. *Nano Lett.* 2013;13(11):5147–5152.
188. Bettinger T, Tranquart F. Design of Microbubbles for Gene/Drug Delivery. *Adv. Exp. Med. Biol.* 2016;880(Chapter 11):191–204.
189. Wang P, Yin T, Li J, et al. Ultrasound-responsive microbubbles for sonography-guided siRNA delivery. *Nanomedicine*. 2015.
190. Xiong X, Sun Y, Sattiraju A, et al. Remote spatiotemporally controlled and biologically selective permeabilization of blood-brain barrier. *J Control Release*. 2015;217:113–120.
191. Engler C, Marillonnet S. Golden Gate Cloning. *DNA Cloning and Assembly Methods*. 2013;1116(Chapter 9):119–131.
192. Ernst OP, Lodowski DT, Elstner M, et al. Microbial and animal rhodopsins: structures, functions, and molecular mechanisms. *Chem. Rev.* 2014;114(1):126–163.
193. Hofmann L, Palczewski K. The G protein-coupled receptor rhodopsin: a historical perspective. *Methods Mol. Biol.* 2015;1271(Chapter 1):3–18.
194. Smith RL, Sivaprasad S, Chong V. Retinal Biochemistry, Physiology and Cell Biology. *Dev Ophthalmol*. 2016;55:18–27.
195. Invergo BM, Montanucci L, Laayouni H, Bertranpetit J. A system-level, molecular evolutionary analysis of mammalian phototransduction. *BMC Evol. Biol.* 2013;13(1):52.
196. Imamoto Y, Shichida Y. Cone visual pigments. *Biochim. Biophys. Acta*. 2014;1837(5):664–673.
197. Chabre M. Visual Rhodopsin and Phototransduction in the Vertebrate Retina. *Trends in Photobiology*. 1982;(Chapter 34):399–412.
198. Lamb TD. Evolution of phototransduction, vertebrate photoreceptors and retina. *Prog Retin Eye Res*. 2013;36:52–119.
199. Terakita A. The opsins. *Genome Biol.* 2005;6(3):213.
200. Koyanagi M, Takada E, Nagata T, Tsukamoto H, Terakita A. Homologs of vertebrate Opn3 potentially serve as a light sensor in nonphotoreceptive tissue. *Proc. Natl. Acad. Sci. U.S.A.* 2013;110(13):4998–5003.
201. Nissilä J, Mänttäri S, Särkioja T, et al. Encephalopsin (OPN3) protein abundance in the adult mouse brain. *J. Comp. Physiol. A Neuroethol. Sens. Neural. Behav. Physiol.* 2012;198(11):833–839.

202. Bailes HJ, Lucas RJ. Human melanopsin forms a pigment maximally sensitive to blue light (λmax ≈ 479 nm) supporting activation of G(q/11) and G(i/o) signalling cascades. *Proc. Biol. Sci.* 2013;280(1759):20122987–20122987.
203. Chew KS, Schmidt TM, Rupp AC, Kofuji P, Trimarchi JM. Loss of gq/11 genes does not abolish melanopsin phototransduction. *PLoS ONE*. 2014;9(5):e98356.
204. Sexton T, Buhr E, Van Gelder RN. Melanopsin and mechanisms of non-visual ocular photoreception. *J. Biol. Chem.* 2012;287(3):1649–1656.
205. Bertolesi GE, Hehr CL, McFarlane S. Melanopsin photoreception in the eye regulates light-induced skin colour changes through the production of α-MSH in the pituitary gland. *Pigment Cell Melanoma Res*. 2015;28(5):559–571.
206. Díaz NM, Morera LP, Guido ME. Melanopsin and the Non-visual Photochemistry in the Inner Retina of Vertebrates(†). *Photochem. Photobiol.* 2015;n/a–n/a.
207. Sikka G, Hussmann GP, Pandey D, et al. Melanopsin mediates light-dependent relaxation in blood vessels. *Proc. Natl. Acad. Sci. U.S.A.* 2014;111(50):17977–17982.
208. Yamashita T, Ohuchi H, Tomonari S, et al. Opn5 is a UV-sensitive bistable pigment that couples with Gi subtype of G protein. *Proc. Natl. Acad. Sci. U.S.A.* 2010;107(51):22084–22089.
209. Yamashita T, Ono K, Ohuchi H, et al. Evolution of mammalian Opn5 as a specialized UV-absorbing pigment by a single amino acid mutation. *J. Biol. Chem.* 2014;289(7):3991–4000.
210. Sugiyama T, Suzuki H, Takahashi T. Light-induced rapid Ca^{2+} response and MAPK phosphorylation in the cells heterologously expressing human OPN5. *Scientific Reports*. 2014;4:5352.
211. Buhr ED, Yue WWS, Ren X, et al. Neuropsin (OPN5)-mediated photoentrainment of local circadian oscillators in mammalian retina and cornea. *Proc. Natl. Acad. Sci. U.S.A.* 2015;112(42):13093–13098.
212. Kojima D, Mori S, Torii M, et al. UV-sensitive photoreceptor protein OPN5 in humans and mice. *PLoS ONE*. 2011;6(10):e26388.
213. Sun H, Gilbert DJ, Copeland NG, Jenkins NA, Nathans J. Peropsin, a novel visual pigment-like protein located in the apical microvilli of the retinal pigment epithelium. *Proceedings of the National Academy of Sciences*. 1997;94(18):9893–9898.
214. Rivolta C, Berson EL, Dryja TP. Mutation screening of the peropsin gene, a retinal pigment epithelium specific rhodopsin homolog, in patients with retinitis pigmentosa and allied diseases. *Mol. Vis.* 2006;12:1511–1515.
215. Ksantini M, Sénéchal A, Humbert G, Arnaud B, Hamel CP. RRH, encoding the RPE-expressed opsin-like peropsin, is not mutated in retinitis pigmentosa and allied diseases. *Ophthalmic Genet.* 2007;28(1):31–37.
216. Maeda T, Van Hooser JP, Driessen CAGG, et al. Evaluation of the role of the retinal G protein-coupled receptor (RGR) in the vertebrate retina in vivo. *J. Neurochem.* 2003;85(4):944–956.
217. Wenzel A, Oberhauser V, Pugh EN, et al. The retinal G protein-coupled receptor (RGR) enhances isomerohydrolase activity independent of light. *Journal of Biological Chemistry*. 2005;280(33):29874–29884.
218. Hao W, Chen P, Fong HK. Analysis of chromophore of RGR: retinal G-protein-coupled receptor from pigment epithelium. *Meth. Enzymol.* 2000;316:413–422.
219. Kim J-M, Hwa J, Garriga P, et al. Light-Driven Activation of β 2-Adrenergic Receptor Signaling by a Chimeric Rhodopsin Containing the β 2-Adrenergic Receptor Cytoplasmic Loops †. *Biochemistry*. 2005;44(7):2284–2292.
220. Airan RD, Thompson KR, Fenno LE, Bernstein H, Deisseroth K. Temporally precise in vivo control of intracellular signalling. *Nature*. 2009;458(7241):1025–1029.
221. Oh E, Maejima T, Liu C, Deneris E, Herlitze S. Substitution of 5-HT1A receptor signaling by a light-activated G protein-coupled receptor. *J. Biol. Chem.* 2010;285(40):30825–30836.
222. Ong W-K, Chen H-F, Tsai C-T, et al. The activation of directional stem cell motility by green light-emitting diode irradiation. *Biomaterials*. 2013;34(8):1911–1920.
223. Ye H, Daoud-El Baba M, Peng R-W, Fussenegger M. A synthetic optogenetic

transcription device enhances blood-glucose homeostasis in mice. *Science*. 2011;332(6037):1565–1568.
224. Bailes HJ, Zhuang L-Y, Lucas RJ. Reproducible and sustained regulation of Gαs signalling using a metazoan opsin as an optogenetic tool. *PLoS ONE*. 2012;7(1):e30774.
225. Nancy. Computational Predictionof the Coupling Specificityof G Protein-Coupled Receptors. 2007;1–10.
226. Edgar RC. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics*. 2004;5(1):113.
227. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*. 2004;32(5):1792–1797.
228. Golubchik T, Wise MJ, Easteal S, Jermiin LS. Mind the gaps: evidence of bias in estimates of multiple sequence alignments. *Mol. Biol. Evol.* 2007;24(11):2433–2442.
229. Mazina O, Allikalt A, Heinloo A, et al. cAMP assay for GPCR ligand characterization: application of BacMam expression system. *Methods Mol. Biol.* 2015;1272(Chapter 5):65–77.
230. Williams C. cAMP detection methods in HTS: selecting the best from the rest. *Nature Reviews Drug Discovery*. 2004;3(2):125–135.
231. Gabriel D, Vernier M, Pfeifer MJ, et al. High throughput screening technologies for direct cyclic AMP measurement. *Assay Drug Dev Technol*. 2003;1(2):291–303.
232. Chambers C, Smith F, Williams C, et al. Measuring intracellular calcium fluxes in high throughput mode. *Comb. Chem. High Throughput Screen.* 2003;6(4):355–362.
233. Zhang Y, Kowal D, Kramer A, Dunlop J. Evaluation of FLIPR Calcium 3 Assay Kit--a new no-wash fluorescence calcium indicator reagent. *J Biomol Screen*. 2003;8(5):571–577.
234. Yun C-O, Cho E-A, Song J-J, et al. dl-VSVG-LacZ, a vesicular stomatitis virus glycoprotein epitope-incorporated adenovirus, exhibits marked enhancement in gene transduction efficiency. *Hum. Gene Ther.* 2003;14(17):1643–1652.
235. Pingoud A, Fuxreiter M, Pingoud V, Wende W. Type II restriction endonucleases: structure and mechanism. *CMLS, Cell. Mol. Life Sci.* 2005;62(6):685–707.
236. Engler C, Gruetzner R, Kandzia R, Marillonnet S. Golden Gate Shuffling: A One-Pot DNA Shuffling Method Based on Type IIs Restriction Enzymes. *PLoS ONE*. 2009;4(5):e5553.
237. Allard S, Kopish K. Luciferase reporter assays: Powerful, adaptable tools for cell biology research. Cell Notes; 2008.
238. Cheng Z, Garvin D, Paguio A, et al. Luciferase Reporter Assay System for Deciphering GPCR Pathways. *Current Chemical Genomics*. 2010;4(1):84–91.
239. Bittinger MA, McWhinnie E, Meltzer J, et al. Activation of cAMP Response Element-Mediated Gene Expression by Regulated Nuclear Transport of TORC Proteins. *Current Biology*. 2004;14(23):2156–2161.
240. Impey S, Smith DM, Obrietan K, et al. Stimulation of cAMP response element (CRE)-mediated transcription during contextual learning. *Nature Neuroscience*. 1998;1(7):595–601.
241. Rao A, Luo C, Hogan PG. TRANSCRIPTION FACTORS OF THE NFAT FAMILY:Regulation and Function. *http://dx.doi.org/10.1146/annurev.immunol.15.1.707*. 2003;15(1):707–747.
242. Lewis RS. Calcium oscillations in T-cells: mechanisms and consequences for gene expression. *Biochemical Society Transactions*. 2003;31(5):925–929.
243. Smrcka AV. G protein signaling: methods and protocols. 2004.
244. Hill S. Reporter-gene systems for the study of G-protein-coupled receptors. *Current Opinion in Pharmacology*. 2001;1(5):526–532.
245. Sekharan S, Morokuma K. Why 11-cis-retinal? Why not 7-cis-, 9-cis-, or 13-cis-retinal in the eye? *J. Am. Chem. Soc.* 2011;133(47):19052–19055.
246. Atwood BK, Lopez J, Wager-Miller J, Mackie K, Straiker A. Expression of G protein-coupled receptors and related proteins in HEK293, AtT20, BV2, and N18 cell lines as revealed by microarray analysis. 12(1):14.

247. Schmidt J, Smith NJ, Christiansen E, et al. Selective orthosteric free fatty acid receptor 2 (FFA2) agonists: identification of the structural and chemical requirements for selective activation of FFA2 versus FFA3. *Journal of Biological Chemistry*. 2011;286(12):jbc.M110.210872–10640.
248. Atwood BK, Lopez J, Wager-Miller J, Mackie K, Straiker A. Expression of G protein-coupled receptors and related proteins in HEK293, AtT20, BV2, and N18 cell lines as revealed by microarray analysis. *BMC Genomics*. 2011;12(1):14.
249. Arnt J, Bøgeso KP, Hyttel J, Meier E. Relative Dopamine D1 and D2 Receptor Affinity and Efficacy Determine Whether Dopamine Agonists Induce Hyperactivity or Oral Stereotypy in Rats. *Pharmacology & Toxicology*. 1988;62(3):121–130.
250. Werner P, Hussy N, Buell G, Jones KA, North RA. D2, D3, and D4 dopamine receptors couple to G protein-regulated potassium channels in Xenopus oocytes. *Molecular Pharmacology*. 1996;49(4):656–661.
251. Pan ZZ, Williams JT. Muscarine hyperpolarizes a subpopulation of neurons by activating an M2 muscarinic receptor in rat nucleus raphe magnus in vitro. *Journal of Neuroscience*. 1994;14(3 Pt 1):1332–1338.
252. BENSON JA. Electrophysiological Pharmacology of the Nicotinic and Muscarinic Cholinergic Responses of Isolated Neuronal Somata from Locust Thoracic Ganglia. *Journal of Experimental Biology*. 1992;170(1):203–233.
253. Schagat T, Paguio A, Kopish K. Normalizing genetic reporter assays: approaches and considerations for increasing consistency and statistical significance. Cell Notes; 2007.
254. Cao J, Panetta R, Yue S, et al. A naive Bayes model to predict coupling between seven transmembrane domain receptors and G-proteins. *Bioinformatics*. 2003;19(2):234–240.
255. Sreekumar KR, Huang Y, Pausch MH, Gulukota K. Predicting GPCR-G-protein coupling using hidden Markov models. *Bioinformatics*. 2004;20(18):3490–3499.
256. Yabuki Y, Muramatsu T, Hirokawa T, Mukai H, Suwa M. GRIFFIN: a system for predicting GPCR-G-protein coupling selectivity using a support vector machine and a hidden Markov model. *Nucleic Acids Research*. 2005;33(Web Server):W148–W153.
257. Sgourakis NG, Bagos PG, Papasaikas PK, Hamodrakas SJ. A method for the prediction of GPCRs coupling specificity to G-proteins using refined profile Hidden Markov Models. *BMC Bioinformatics*. 2005;6(1):104–12.
258. Sgourakis NG, Bagos PG, Papasaikas PK, Hamodrakas SJ. A method for the prediction of GPCRs coupling specificity to G-proteins using refined profile Hidden Markov Models. *BMC Bioinformatics*. 2005;6(1):104–12.
259. Ono T, Hishigaki H. Prediction of GPCR-G Protein Coupling Specificity Using Features of Sequences and Biological Functions. *Genomics, Proteomics & Bioinformatics*. 2006;4(4):238–244.
260. GUO Y-Z, LI M-L, WANG K-L, et al. Fast Fourier Transform-based Support Vector Machine for Prediction of G-protein Coupled Receptor Subfamilies. *Acta Biochim Biophys Sinica*. 2005;37(11):759–766.
261. Sander JD, Joung JK. CRISPR-Cas systems for editing, regulating and targeting genomes. *Nature biotechnology*. 2014.
262. Shalem O, Sanjana NE, Hartenian E, et al. Genome-Scale CRISPR-Cas9 Knockout Screening in Human Cells. *Science*. 2014;343(6166):84–87.
263. Niedernberg A, Tunaru S, Blaukat A, Ardati A, Kostenis E. Sphingosine 1-phosphate and dioleoylphosphatidic acid are low affinity agonists for the orphan receptor GPR63. *Cell. Signal.* 2003;15(4):435–446.
264. Thathiah A, De Strooper B. The role of G protein-coupled receptors in the pathology of Alzheimer's disease. *Nature Reviews Neuroscience*. 2011;12(2):73–87.
265. Rourke JL, Dranse HJ, Sinal CJ. CMKLR1 and GPR1 mediate chemerin signaling through the RhoA/ROCK pathway. *Mol. Cell. Endocrinol.* 2015;417(C):36–51.
266. Yang C-R, Wei Y, Qi S-T, et al. The G Protein Coupled Receptor 3 Is Involved in cAMP and cGMP Signaling and Maintenance of Meiotic Arrest in Porcine Oocytes. *PLoS ONE*. 2012;7(6):.
267. Tobo M, Tomura H, Mogi C, et al. Previously postulated “ligand-independent” signaling

of GPR4 is mediated through proton-sensing mechanisms. *Cell. Signal.* 2007;19(8):1745–1753.
268. Ludwig M-G, Vanek M, Guerini D, et al. Proton-sensing G-protein-coupled receptors. *Nature*. 2003;425(6953):93–98.
269. Liu J-P, Nakakura T, Tomura H, et al. Each one of certain histidine residues in G-protein-coupled receptor GPR4 is critical for extracellular proton-induced stimulation of multiple G-protein-signaling pathways. *Pharmacol. Res.* 2010;61(6):499–505.
270. Takenouchi R, Inoue K, Kambe Y, Miyata A. N-arachidonoyl glycine induces macrophage apoptosis via GPR18. *Biochemical and Biophysical Research Communications*. 2012;418(2):366–371.
271. Kohno M, Hasegawa H, Inoue A, et al. Identification of N-arachidonylglycine as the endogenous ligand for orphan G-protein-coupled receptor GPR18. *Biochemical and Biophysical Research Communications*. 2006;347(3):827–832.
272. Bresnick JN, Skynner HA, Chapman KL, et al. Identification of signal transduction pathways used by orphan g protein-coupled receptors. *Assay Drug Dev Technol*. 2003;1(2):239–249.
273. Rompler H, Schulz A, Pitra C, Coop G. The rise and fall of the chemoattractant receptor GPR33. *Journal of Biological …*. 2005.
274. Brown AJ, Goldsworthy SM, Barnes AA, et al. The Orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. *Journal of Biological Chemistry*. 2003;278(13):11312–11319.
275. Ryberg E, Larsson N, Sjögren S, et al. The orphan receptor GPR55 is a novel cannabinoid receptor. *Br J Pharmacol*. 2007;152(7):1092–1101.
276. Yang M, Mailhot G, Birnbaum MJ, et al. Expression of and role for ovarian cancer G-protein-coupled receptor 1 (OGR1) during osteoclastogenesis. *Journal of Biological Chemistry*. 2006;281(33):23598–23605.
277. Liu J-P, Komachi M, Tomura H, et al. Ovarian cancer G protein-coupled receptor 1-dependent and -independent vascular actions to acidic pH in human aortic smooth muscle cells. *Am. J. Physiol. Heart Circ. Physiol.* 2010;299(3):H731–42.
278. Xu Y. Sphingosylphosphorylcholine and lysophosphatidylcholine: G protein-coupled receptors and receptor-mediated signal transduction. *Biochim. Biophys. Acta*. 2002;1582(1-3):81–88.
279. Jones PG, Nawoschik SP, Sreekumar K, et al. Tissue distribution and functional analyses of the constitutively active orphan G protein coupled receptors, GPR26 and GPR78. *Biochim. Biophys. Acta*. 2007;1770(6):890–901.
280. Wang X, Sumida H, Cyster JG. GPR18 is required for a normal CD8 intestinal intraepithelial lymphocyte compartment. *Journal of Experimental Medicine*. 2014;211(12):2351–2359.
281. Takenouchi R, Inoue K, Kambe Y, Miyata A. N-arachidonoyl glycine induces macrophage apoptosis via GPR18. *Biochemical and Biophysical Research Communications*. 2012;418(2):366–371.
282. Becker AM, Callahan DJ, Richner JM, et al. GPR18 Controls Reconstitution of Mouse Small Intestine Intraepithelial Lymphocytes following Bone Marrow Transplantation. *PLoS ONE*. 2015;10(7):e0133854.
283. Hirasawa A, Hara T, Katsuma S, Adachi T, Tsujimoto G. Free Fatty Acid Receptors and Drug Discovery. *Biological and Pharmaceutical Bulletin*. 2008;31(10):1847–1851.
284. Andradas C, Caffarel MM, Perez-Gomez E, et al. The orphan G protein-coupled receptor GPR55 promotes cancer cell proliferation via ERK. *Oncogene*. 2011;30(2):245–252.
285. Horiguchi K, Higuchi M, Yoshida S, et al. Proton receptor GPR68 expression in dendritic-cell-like S100β-positive cells of rat anterior pituitary gland: GPR68 induces interleukin-6 gene expression in extracellular acidification. *Cell Tissue Res*. 2014;358(2):515–525.