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Conserved 2nd Residue of Helix 8 of GPCR May Confer the Subclass-Characteristic and Distinct Roles through a Rapid Initial Interaction with Specific G Proteins

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Abstract: To obtain a systematic view of the helix-8-second residue responsible for G protein-coupled receptor (GPCR)–G protein initial specific interactions, 786 human GPCRs were subclassified based on the pairs of agonist groups and target G proteins and compared with their conserved second residue of helix 8. Of 314 non-olfactory and deorphanized GPCRs, 273 (87%) conserved single amino acids in the subclasses, while 93 (58%) of the 160 subclasses possessed only a single GPCR member. Class B, C, Frizzled, and trace amine-associated GPCRs demonstrated 100% conservation, whereas class I and II olfactory and vomeronasal 1 receptors demonstrated much lower rates of conservation (20–47%). These conserved residues are characteristic of GPCR classes and G protein subtypes and confer their functionally-distinct roles.

Keywords: G protein-coupled receptor; G protein subtypes; human; classification; initial specific interaction; helix 8; hydrophobic core

1. Introduction

In humans, nearly 800 G protein-coupled receptors (GPCRs) detect various extracellular physiological or environmental signal molecules. These range widely from atomic ions to structural features of proteins. Activated GPCRs activate one or a few of the 16 G proteins for one or more distinct cellular responses, leading to regulations of various internal physiological systems such as cardiovascular, neural, immune, sensory, hormonal, and differentiation systems [1]. GPCRs are typically classified into eight classes: class A (279 non-olfactory members, 52 class I and 333 class II olfactory receptors (ORs), 6 trace amine-associated receptors (TAARs)), class B (15 members), class C (23 members), adhesion class (33 members), Frizzled class (11 members), vomeronasal type 1 (VN1, 5 members), Taste2 (TAS2, 25 members), and the other GPCRs (4 members) [1,2]. Evolutionarily, the divergence of class A GPCRs is expanded in multicellular animals, whereas unicellular organisms mainly possess class B and class C GPCRs [3]. G proteins are grouped into four classes: G_s class (G_s, G_{olf}), $G_{i/o}$ class (G_{i1}, G_{i2}, G_{i3}, G_{t1}, G_{t2}, G_{t3}, G_o, G_z), $G_{q/11}$ class (G_q, G_{11}, G_{14}, G_{15}), and $G_{12/13}$ class (G_{12}, G_{13}) [3], and contrastingly, unicellular organisms have representatives of all four human G protein classes [3]. The crystal structures of the active-state rhodopsin and β_2 adrenergic receptor (β_2AdR) led to the discovery of the common rearrangement mechanism behind the intramolecular interaction of GPCR during its activation [4–6]. However, the molecular mechanism underlying specific interactions between GPCRs and G protein remains unclear, except for the selectivity barcode of 25 amino acids in G proteins [3].

A systematic analysis of a chimeric G protein and scanning mutagenesis of a GPCR has shed light on responsible residues for the specific interaction. The replacement of the non-olfactory $G\alpha_{15}$ C-terminal

six amino acids, ^{369}DEINLL, with the corresponding Gα_{olf}, ^{376}KQYELL, improved the interaction specificity between OR-S6 and Gα_{15} [7]. This chimeric G protein mediated a more rapid (2.2-fold) and robust (1.7-fold) Ca^{2+} response in a HEK293 functional expression system [7,8]. Regarding responsible residues of the GPCR, the second residue of helix 8 of OR-S6 was identified, by observing the complete loss of improved response dynamics in an alanine-scanning and charge-altering mutagenesis of OR-S6 helix 8 with the chimeric G$\alpha_{15\text{-olf}}$. The homology modeling indicates that the specific interaction between OR-S6 and G$\alpha_{15\text{-olf}}$ is based on the stabilized intracellularly-superficial configuration of the helix-8-second residue by the hydrophobic core between helix 8 and transmembrane domain 1–2 (TM1–2) [8]. Then, the initial, transient, and specific interaction between a GPCR, OR-S6, helix-8-second residue (Glu) and G$\alpha_{15\text{-olf}}$ C-terminal sixth residue (Lys) was predicted and supported by a high conservation of helix-8-second residues in the GPCR subclasses based on pairs of agonist groups and G protein subtypes for 178 non-olfactory GPCRs [9]. Moreover, its functional importance is supported by an almost identical class-dependent occupancy of helix-8-second-Glu class I and II ORs in humans and mice [9]. Moreover, a transition step model from an inactive state to a stable interaction via an initial, transient, and specific inter-helical interaction was proposed (Figure 1) [10]. The model starts with the inactive-state crystal structure of β_2AdR and ends with its active-state crystal structure of the complex with the extended interactions between GPCR and G protein through possible intermediate processes, which facilitate breaking some of the inactive-state intra-molecular interactions.

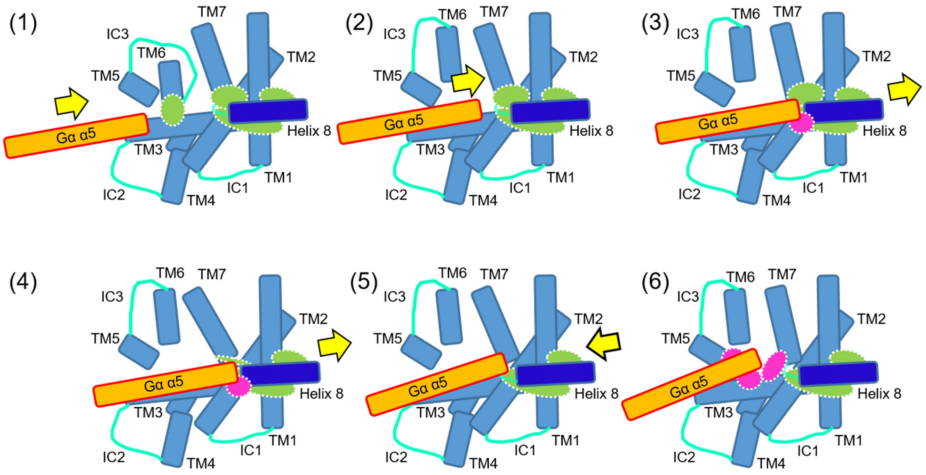

Figure 1. A transition model of multistep interactions between GPCR and G protein [10]. The cytoplasmic view of a possible sequential interaction process is shown. Intramolecular interactions (green closed circles) and intermolecular interactions (magenta closed circles) are broken, or maintained, or formed.

In the present study, to obtain a systematic view of the helix-8-second residue responsible for the GPCR–G protein initial specific interactions, our previous analysis of conserved helix-8-second residues was expanded to 786 human GPCRs. The conserved helix-8-second residues are characteristic of GPCR classes and specific G protein subtypes. These results suggest that the conserved helix-8-second residues confer functionally distinct roles in parallel GPCR signaling. The initial and subclass-characteristic transient process can be a potential drug target for specific GPCR-regulated signaling pathways.

2. Results and Discussion

2.1. High Conservation of Helix-8-Second Residues of GPCRs in Subclasses Except for ORs and VN1 Receptors

In the Supplementary Materials, the sequences of TM7 NPxxY or corresponding motifs and adjacent helix 8 of GPCRs and their target G proteins are shown for non-olfactory and deorphanized class A GPCRs (204 members: 194 and 10 GPCRs with identified and not identified G proteins,

respectively, Figure S1), class B GPCRs (15 members, Figure S2), deorphanized class C GPCRs (15 members, Figure S3), adhesion class GPCRs (33 members, Figure S4), Frizzled class GPCRs (11 members, Figure S5), VN1 receptors (5 members) and other GPCRs (4 members, Figure S6), TAS2 GPCRs (25 members, Figure S7), non-olfactory and orphan class A GPCRs (75 members, Figure S8), and orphan class C GPCRs (8 members, Figure S9). They were subclassified based on GPCR classes and pairs of agonist group and G protein subtypes (Tables S1–S5).

The rates of GPCRs with a conserved helix-8-second residue in each subclass are summarized in Table 1, except for 75 members of non-olfactory and orphan class A GPCRs and eight members of class C GPCRs. Of the 314 non-OR and deorphanized GPCRs (160 subclasses), 273 (87%) (135 subclasses; 84%) conserved single helix-8-second residues in the subclasses. Class B, C, and Frizzled GPCRs and TAARs demonstrated 100% conservation. The high conservation of GPCR helix-8-second residues suggests their important subclass-dependent role in GPCR signaling.

Why does the GPCR signaling system require the conserved helix-8-second residues? As described previously [9,10], both GPCRs and G proteins are activated in two-step processes, which are an initial, transient, and specific interaction and a subsequent GPCR-common and stable interaction. The initial interaction is likely an activation rapidity determinant, whereas the stable one is likely formed in an initial interaction-dependent manner. The main determinant of GPCR signaling is likely agonist affinities of GPCRs. In addition, when agonist–GPCR binding affinities are similarly high, GPCR–G protein interaction specificities must be critical for rapid and robust cellular responses. Although only one scanning mutagenesis analysis has concluded that the helix-8-second residue is a determinant for the initial, transient, and specific interaction of a GPCR and a chimeric G protein, the high conservation of the helix-8-second residue in the deorphanized GPCR subclasses strongly supports that a single residue at the second position of helix 8 governs cellular response rapidity via GPCR–G protein initial interaction specificities. In other words, the conserved helix-8-second residue could simply assure an agonist binding affinity-dependent cellular response in parallel GPCR signaling pathways via a uniform activation rapidity of a target G protein.

Table 1. Rate of human GPCRs with conserved helix-8-second residue in agonist–G protein pair-based subclasses.

GPCR Class	Class A				Class B GPCRs	Class C GPCRs	Adhesion Class GPCRs	Frizzled Class GPCRs	VN1 GPCRs	TAS2 GPCRs	Non-OR GPCRs
	Non-Olfactory Class A GPCRs	Class-I ORs	Class-II ORs	TAARs							
All GPCRs † (subclasses)	204 (117)	52 (1)	333 (1)	6 (1)	15 (10)	15 (7)	33 (16)	11 (7)	5 (1)	25 (1)	314 (160)
Conserved GPCRs ‡ (subclasses)	179 (98)	12 (0)	156 (0)	6 (1)	15 (10)	14 (6)	28 (13)	11 (7)	1 (0)	19 (0)	273 (135)
Conserved GPCR rate (subclass rate)	88% (84%)	23% (0%)	47% (0%)	100% (100%)	100% (100%)	93% (86%)	85% (81%)	100% (100%)	20% (0%)	76% (0%)	87% (84%)

GPCR, G protein-coupled receptor; OR, olfactory receptor; TAAR, trace amine-associated receptor; VN1, vomeronasal type 1; TAS2, Taste2. Human GPCRs were subclassified by pairs of agonist group and G-protein subtypes. Orphan class A and class C GPCRs † were excluded. Helix-8-2nd-Glu class I and II ORs were counted for single-amino acid-conserved GPCR ‡.

2.2. GPCR Class- and G Protein Subtype-Characteristics of Conserved Helix-8-Second Residues

In contrast, class I and II ORs and VN1Rs demonstrated much lower conservation rates (20–47%) of helix-8-second residues (Table 1). These differences are attributable to a genetic origin and species dependency. In humans, helix-8-second-Glu ORs are 23% and 47% in classes-I and class-II ORs, respectively, whereas helix-8-second Asp ORs are 0% and 42%, respectively, with high cross-species conservation between human and mouse [9,10]. However, pheromone receptors, human VN1Rs and murine *m*Vmn1rs, form a more species-specific family (Table 2), consistent with the previous report [11].

In mice, 43% of 112 *m*Vmn1rs, which interact with G_{i2}, conserved Arg and no helix-8-second-Glu, Gln, or Asp *m*Vmn1rs, whereas human VN1Rs equally conserved Arg (20%), His (20%), and Gln (20%) (Table 2, Figure S6). Notably, 117 *m*Vmn1rs, which showed no characteristic features of helix 8, were excluded. Considering the lack of helix-8-second-Arg ORs and the predicted initial, transient, and specific, inter-helical, and ionic interaction between GPCR helix-8-second-Glu and G_{olf} C-terminal sixth Arg, the positively-charged helix-8-second-Arg would specifically interact with G_{i2} C-terminal fifth Asp (Figure S10). Similarly, four subclasses of chemokine receptors, which conserve Lys (60–100%), would also specifically interact with G_i C-terminal fifth Asp or Glu (Table S1, Figures S1 and S10). Moreover, conserved Lys in bitter tastant TAS2 receptors (76%) indicates an initial and specific interaction between GPCR helix-8-second Lys and G_{t3} C-terminal fifth Asp (Table 2, Figure S10).

Thus, the present analysis complemented the results from a previous study [10], where highly-conserved helix-8-second residues are characteristic of GPCR subclasses, i.e., characteristic of GPCR classes and G protein subtypes (Table 2). Markedly, Trp is conserved at the second position of helix 8 only in TAARs, which mediate aversive responses to odors, in both humans and mice [10], but not in all the other GPCRs. Non-olfactory class-A GPCRs similarly conserved helix-8-second Glu (16%), Asp (13%), Asn (15%), and Lys (14%). This contrasts with the uneven rates of helix-8-second Glu, Gln, and Asp in class I and II ORs (Table 2). Moreover, the highly-conserved helix-8-second residues were Glu (100%) for class B GPCRs, Asn (60%) for class C GPCRs, Glu (24%) and Lys (27%) for adhesion class GPCRs, and Lys (91%) for Frizzled class GPCRs, whereas no characteristic residues were observed for orphan class A and C GPCRs and G-protein-unknown, non-olfactory class A GPCRs (Table 2, Figures S8 and S9). In non-OR GPCRs, the most conserved helix-8-second residue was Lys (for 75 GPCRs = 82 − 1 − 6), consistent with a high rate of 11/16 G proteins for negatively-charged Asp or Glu at the C-terminal fifth or sixth position of α5 compared to 5/16 for non-charged residues.

To shed light on the initial interaction specificities between GPCRs and G proteins, the differences in conserved helix-8-second residues between G protein subtypes were further analyzed in non-olfactory and deorphanized class A, class B, and class C GPCRs. The GPCRs with each helix-8-second residue were summed for combinations of target G protein subtypes in each class (Table S6). In G protein subtypes, the conserved helix-8-second residues were GPCR class-dependent and characteristic of G_s (Glu (33%) and Asp (38%) in class A vs. Glu (100%) in class B), $G_{i/o}$ (Lys (25%) and Asn (23%) in class A vs. Asn (100%) class C), and $G_{q/11}$ (Glu (19%), Arg (14%), His (14%), and Ser (14%) in class A vs. Glu (40%) and Asn (40%) in class C).

Table 2. Classification of olfactory receptors and other GPCRs by helix-8-second residues and subtypes of G proteins (modified from [9,10]).

GPCRs and Their Rates	Helix-8 Second Residue										2nd Residue of Helix 8 (GPCR Number)	NPxxY Motif
	All	Glu	Gln	Asp	Asn	His	Lys	Arg	Trp	Others		
non-olfactory class A GPCRs'	194	**31**	5	**26**	**30**	7	**28**	19	**0**	48	S(15), T(12), G(6), A(5), P(4), I(2), L(1), V(1), F(1), no h8(1)	(N/D)PxxY, (N/D)PxxF
rate (misc)	100%	**16%**	3%	**13%**	**15%**	4%	**14%**	10%	**0%**	25%		
class-I ORs'	52	**12**	**36**	0	0	1	1	0	**0**	2	T(1), P(1)	NPxxY
rate (G_{olf})	100%	**23%**	**69%**	0%	0%	2%	2%	0%	**0%**	4%		
class-II ORs'	333	**156**	22	**139**	1		6	0	**0**	7	A(3), V(1), T(1), S(1), M(1)	
rate (G_{olf})	100%	**47%**	7%	**42%**	0%	1%	2%	0%	**0%**	2%		
TAARs'	6	0	0	0	0	0	0	0	**6**	0		
rate	100%	0%	0%	0%	0%	0%	0%	0%	**100%**	0%		
class B GPCRs'	15	**15**	0	0	0	0	0	0	**0**	0		V(A/S)xxY
rate (G_s, $G_s > G_{q/11}$, –)	100%	**100%**	0%	0%	0%	0%	0%	0%	**0%**	0%		
class C GPCRs'	15	3	0	2	**9**	0	0	0	**0**	1	G(1)	PKCYxY, VYIIxF, IYIILF
rate ($G_{i/o}$, $G_{q/11}$, –)	100%	20%	0%	13%	**60%**	0%	0%	0%	**0%**	7%		
adhesion class GPCRs'	33	**8**	5	4	2	0	**9**	0	**0**	5	S(1), T(1), C(1), P(1), no helix 8(1)	Fx(V/I)xxx(H/Y)C, xFIFxF(H/Y)C, LFIFLx(H/Y)C
rate ($G_{q/11}$, G_s, $G_{12/13}$)	100%	**24%**	15%	12%	6%	0%	**27%**	0%	**0%**	15%		
Frizzled class GPCRs'	11	0	0	0	0	0	**10**	0	**0**	1	A(1)	ITSxxWI, TGIAMxW
rate ($G_{i/o}$, $G_{q/11}$, G_s, $G_{12/13}$)	100%	0%	0%	0%	0%	0%	**91%**	0%	**0%**	9%		
vomeronasal 1 Rs'	5	0	1	0	0	1	0	1	**0**	2	S(2), no helix 8 in R1(H), R2(S) & R3(R)?	SPxxL
rate (G_{i2})	100%	0%	20%	0%	0%	20%	0%	20%	**0%**	40%		
murine vomeronasal 1 Rs'	112	0	0	0	0	11	15	**48**	**0**	38	L(12), T/S(7+6), I(3), F/M/Y(3+2+2), P(2)	TPLVQ, TSYSI, SPLVF, SPxVL, ITxII
rate (G_{i2})	100%	0%	0%	0%	0%	10%	13%	**43%**	**0%**	34%		
murine vomeronasal 1 Rs'	117	0	0	0	0	0	0	0	**0**	117	no helix 8	no characteristic features of helix 8
rate (G_{i2})	100%	0%	0%	0%	0%	0%	0%	0%	**0%**	100%		
Bitter tastant TAS2 Rs'	25	0	0	0	0	0	**19**	2	**0**	4	G(2), T(2)	H(S/P)xIL
rate (G_{t3})	100%	0%	0%	0%	0%	0%	**76%**	8%	**0%**	16%		

Table 2. *Cont.*

GPCRs and Their Rates	Helix-8 Second Residue										2nd Residue of Helix 8 (GPCR Number)	NPxxY Motif
	All	**Glu**	**Gln**	**Asp**	**Asn**	**His**	**Lys**	**Arg**	**Trp**	**Others**		
non-olfactory class A GPCRs′	10	0	1	1	1	0	1	1	**0**	5	T(2), S(1), A(1), no helix 8(1)	(N/S/T)PxxY, no NPxxY
rate (no identified G proteins)	100%	0%	10%	10%	10%	0%	10%	10%	**0%**	50%		
orphan class A GPCRs′	75	**9**	**6**	**5**	**6**	**6**	**6**	**4**	**0**	**33**	G(6), T(7), Y(1), V(2), S(8), P(4), A(2), F(1), no helix 8(2)	(N/D)PxxY, (N/D)PxxF
rate ($G_{q/11}$, $G_{i/o}$, G_s, –)	100%	**12%**	**8%**	**7%**	**8%**	**8%**	**8%**	**5%**	**0%**	**44%**		
orphan class C GPCRs′	8	2	1	0	0	0	1	0	**0**	4	S(1),A(2), no helix 8(1)	PKCYxI, TTTxxL, no conserved motif
rate ($G_{i/o}$)	100%	25%	13%	0%	0%	0%	13%	0%	**0%**	50%		
other GPCRs′	4	0	0	0	0	0	1	1	**0**	2	S(1), A(1)	SPxxL, TLxxF, LPxxL, SLxxY, or NCxxF
rate (?)	100%	0%	0%	0%	0%	0%	25%	25%	**0%**	50%		
all human GPCRs′	786	236	77	177	49	16	82	28	6	114		
rate (misc)	100%	30%	10%	23%	6%	2%	10%	4%	1%	15%		

Common to class A and B GPCRs likely coupling to G_s, a highly-conserved helix-8-second residue was the negatively-charged Glu, supporting the initial and specific interaction with the positively-charged Arg at the sixth position of the C-terminal. Similarly, Glu was highly and commonly conserved for class A and C GPCRs, which interact only with $G_{q/11}$, suggesting an initial, transient, and specific interaction with G protein C-terminal sixth Lys. However, class A and C GPCRs interacting only with $G_{i/o}$ commonly conserved non-charged polar helix-8-second-Asn, a specific interaction partner of which was not predicted. In addition to Asn, class A GPCRs for $G_{i/o}$ similarly conserved positively-charged Lys, which was able to form initially a specific interaction with the C-terminal fifth negatively-charged Asp or Glu in a manner similar to that of TAS2 GPCRs.

Notably, based on the stable interaction between M3R helix-8-first Lys and G_q loop G.h4s6.12 Asp (common Gα numbering system) [12,13], a non-specific or less specific, stable, and loop-helical interaction between GPCR helix-8-first Lys and G protein G.h4s6.10 or G.h4s6.12 Asp was predicted [10]. This is supported by the charge-altering mutant OR-S6-impaired response rapidity [8–10]. In a future study, a scanning mutagenesis analysis would validate this model by running a comparison between the relative contributions of G protein C-terminal sixth and fifth residues to the initial interactions with these GPCR helix-8-second residues.

2.3. Key GPCRs for the Determination of Detected Physiological and/or Biological Information in Parallel GPCR Signaling

The principles are generally simple, but hidden by complicated phenomena under additional heterogeneous conditions. A single residue-determined GPCR–G protein interaction specificity is a potential candidate for the principle in parallel GPCR signaling due to its simplicity. Highly-conserved helix-8-second Glu was overlapped between GPCRs for G_s and $G_{q/11}$. However, the cell type-specific expression of the G protein subtype could prevent the overlapping of helix-8-second Glu from mediating cross-talk between parallel GPCR–G_s and $G_{q/11}$ signaling pathways. If this is the case, the GPCR subclass-characteristic, i.e., specific G protein-characteristic helix-8-second residues, would strengthen the transition step model of the GPCR–G protein initial, transient, specific, and inter-helical interaction into a common, stable, extended interaction for a single-residue-determined uniform activation rapidity of a target G protein [9,10,14]. The helix-8-second residue could determine within-subclass-distinct functional roles of GPCRs in parallel GPCR signaling.

Next, we determined which GPCR with a conserved helix-8-second residue is key to controlling parallel GPCR-mediated regulations of multiple physiological systems. The GPCRs, which are most sensitive to a given agonist and most specific for a target G protein, most rapidly activate the G protein and subsequently induce the most robust cellular responses. Such a rapid and robust response could be a determinant in the parallel GPCR-mediated regulations or signaling. GPCRs that mediate such determinant responses are defined as key GPCRs. Among the GPCR subclasses, the most difficult question is "Which residue do key ORs conserve at the second position of helix 8?" Considering the specific conditions and evidence for the OR subfamily and the olfactory neural system, the principle would address this question.

The present analysis confirmed that the dual multiple subclasses of the OR subfamily are unique in GPCR subfamilies [9,10]. GPCRs with a rapidly-interacting, subclass-dependent, and highly-conserved helix-8-second residue are likely to be key GPCRs. Glu is the only rapidly-interacting and highly-conserved helix-8-second residue common between class I and II ORs. A series of point mutations at the helix-8-second residue in OR-S6 indicates the importance of the negative charge for cellular response rapidity via the chimeric $G\alpha_{15\text{-}olf}$. Although Asp is also negatively charged, there are no class I helix-8-second-Asp ORs [9,10]. Furthermore, our transition step model of the GPCR–G interaction [10] predicts the advantage of Glu for a rapid activation. In the homology modeling of OR-S6 and the transition step model [9,10], the negatively-charged atom of Glu at the second position of helix 8 is one carbon chain-length closer to the accessing C-terminal region of G protein α5, suggesting a more rapid initiation of the initial interaction between GPCR helix 8 and G protein α5. These differences

and the following architecture of the odor information processing both suggest that helix-8-second-Glu ORs are key ORs rather than auxiliary ORs. The architecture of the odor information processing demonstrates why response rapidity is very important in the olfactory system [9,10,14].

A signal of an odorant is detected in the olfactory sensory neuron (first neuron), which transfers the OR signal to mitral and tufted cells (second neurons) in the first olfactory center via one or two OR-specific relay points. The third neurons of the olfactory pathway are distributed in the second olfactory centers. As one of them, pyramidal cells in the anterior piriform cortex integrate signals from multiple cognate ORs by input synchrony through feedforward inhibition via the more sensitive tufted-cell pathway [15,16]. These integrated signals are characteristic of distinct odors, likely representing elemental odors (corresponding to the R/G and Y/B elemental colors primarily extracted in the visual third neurons under inhibitory conditions) [9,10,14]. Notably, wavelet correlation analysis revealed that input and output signals of the third neurons change in information redundancy [17].

A change in initially-activated key ORs could alter perceived odors in a hierarchical elemental-odor coding manner. The odor mixture-dependent stress relaxation indicates a hierarchy of elemental odors: rose odor > fox-unique 2,4,5-trimethyl thiazoline (TMT) odor > caraway odor [18,19]. The less sensitive key OR for TMT likely provides an explanation for the above and associated results. By the genetic ablation of all dorsal ORs, ΔD mice are unable to recognize fox-unique TMT odor, although they retain the high sensitivity to TMT [20]. The most sensitive OR for TMT odor is helix-8-second-Asp OR [21], while only one helix-8-second-Glu OR, as a candidate of key OR for TMT, is less sensitive than the other three helix-8-second-Asp ORs [10]. The deletion of the less-sensitive key OR results in the impaired recognition of TMT odor and the maintained high sensitivity to TMT via the remaining highly-sensitive helix-8-second-Asp ORs [10]. Early inputs from key ORs to the third neurons in the ventrorostral region of the anterior piriform cortex would coordinate the integration of cognate OR signals for rose odor earlier than those of TMT odor through input synchrony by the feedforward inhibitory signals delivered entirely within the anterior piriform cortex, resulting in the higher ranked hierarchy of rose odor compared to TMT odor [10,14].

In contrast, all human and murine ORs that are most sensitive to an elementally-resistant odor of musk, which is used as base notes in many perfumes, are helix-8-second-Glu ORs [22]. Moreover, the deletion of the most sensitive helix-8-second-Glu OR for R-(−)-carvone and the maintenance of the most sensitive helix-8-second-Glu and R-(−)-/S-(+)-carvone-non-discriminating OR could explain an inability to distinguish between R-(−)- and S-(+)-carvone with a retained high sensitivity to R-(−)-carvone [14]. These results strongly suggest that helix-8-second-Glu ORs are key ORs for the determination of odor representation. Helix-8-second-Gln ORs and helix-8-second-Asp ORs could therefore contribute to odor decoding as auxiliary elemental odors and odor detection sensitizers, respectively, in the brain [9,10].

Thus, conserved helix-8-second residues confer functionally-distinct roles in parallel GPCR signaling. In both ORs and non-OR GPCRs, such as the three adrenergic receptor subclasses, the signal/elemental information hierarchy will be validated in future studies. Analysis of response kinetics using chimeric $G\alpha_{15}$ proteins by replacing the C-terminal six residues of each G protein subtype would be useful to validate the transition step model for rapid and specific activations of GPCRs and G proteins. Moreover, analysis of residues for the extended stable interaction between GPCRs and G proteins using scanning mutagenesis would be required to understand fully the molecular mechanism underlying GPCR–G protein interaction specificities and hierarchical GPCR signal processing.

3. Materials and Methods

3.1. Subclassification of GPCRs

Sequences of TM7 and helix 8 for target G protein subtypes were analyzed for non-olfactory GPCRs (204 deorphanized + 75 orphan members), class B GPCRs (15), class C GPCRs (15 deorphanized + 8 orphan), adhesion class GPCRs (33), Frizzled class GPCRs (11), and other GPCRs (4) in humans

using data from the IUPHAR/BPS Guide to Pharmacology database [1] and its linked webpage at the Universal Protein Resource (https://www.uniprot.org/) or data from our previous papers with some corrections [9,10]. The sequences of an OR (OR2AT4), VN1 receptors (5 VN1Rs and 229 murine mVmn1rs, including 18 predicted murine members), and TAS2 receptors (24) in humans were obtained from the NCBI gene database [2] and its linked webpage at the Universal Protein Resource (https://www.uniprot.org/). The other GPCRs (52 class I and 332 class II ORs, 6 TAARs) were re-used from our previous papers [9,10]. All GPCRs were subclassified based on their classes, agonist groups, and target G proteins.

3.2. Alignment of TM7 NPxxY Motif and Helix 8 of GPCR

The alignment of the TM7 NPxxY motif and helix 8 of GPCRs was manually achieved based on the sequence features observed in our reported homology modeling of OR-S6 [8]. Helix 8 was predicted based on two criteria: (i) hydrophobic residues at the 3rd, 7th, and/or 8th and 10th and/or 11th position of helix 8 in the C-terminal region of the GPCR and (ii) the 2nd residue of helix 8 located at the 7th or 8th position from the last Tyr residue of the NPxxY motif or those of other corresponding and conserved motifs located close to the C-terminus of TM7. Hydrophobic residues for the hydrophobic core between helix 8 and TM1–2 were predicted based on the positions of those in the OR-S6 [8]. Some of the predicted helix 8 and their instability in previously-reported GPCRs [10] were modified in the present study.

Supplementary Materials: Supplementary Materials can be found at http://www.mdpi.com/1422-0067/20/7/1752/s1.

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Conserved Second Residue of Helix 8 of GPCR May Confer Subclass-Characteristic and Distinct Roles through a Rapid Initial Interaction with Specific G Proteins

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Supporting Information

Supplementary Data

Table S1–S5

Fig. S1–S10

Table S1. Classification of class A GPCRs by helix 8-second residues & subtypes of G proteins.

GPCRs	Helix-8 second residue										Predicted Hierarchy,
(signal, G protein subtypes)	all	Glu	Gln	Asp	Asn	His	Lys	Arg	Trp	misc	the 2nd residue, misc.
Rhodopsin/ Opsin1SW/MW/LW Rs	4	0	**4**	0	0	0	0	0	0	0	
(light, G_t)	100%	0%	100%	0%	0%	0%	0%	0%	0%	0%	
Opsin3/4/5 Rs	3	0	0	0	0	0	**3**	0	0	0	OPN3: ligands? G-protein
(light?, $G_{i/o}$)	100%	0%	0%	0%	0%	0%	**100%**	0%	0%	0%	subtypes?
$\beta_{1/2/3}$ Adrenergic Rs	3	0	0	**3**	0	0	0	0	0	0	
(hormone, G_s)	100%	0%	0%	**100%**	0%	0%	0%	0%	0%	0%	
$\alpha_{1A/B/D}$ Adrenergic Rs	3	**3**	0	0	0	0	0	0	0	0	
(hormone, $G_{q/11}$)	100%	**100%**	0%	0%	0%	0%	0%	0%	0%	0%	
$\alpha_{2A/B/C}$ Adrenergic Rs	3	0	0	**3**	0	0	0	0	0	0	
(hormone, $G_{i/o}$)	100%	0%	0%	**100%**	0%	0%	0%	0%	0%	0%	
Dopamine D1/5 Rs	2	0	0	**2**	0	0	0	0	0	0	
(neurotransmitter, G_s)	100%	0%	0%	**100%**	0%	0%	0%	0%	0%	0%	
Dopamine D2/3/4 Rs	3	**3**	0	0	0	0	0	0	0	0	
(neurotransmitter, $G_{i/o}$)	100%	**100%**	0%	0%	0%	0%	0%	0%	0%	0%	
Serotonin 5-$HT_{4/6/7}$ Rs	3	0	0	2	0	0	0	0	0	1	5-HT_6(D)/$_7$(D) >
(neurotransmitter, G_s)	100%	0%	0%	67%	0%	0%	0%	0%	0%	17%	5-HT_4(S) (G_s)
Serotonin 5-$HT_{1A/B/D/E/F/5A}$ Rs	6	1	0	4	1	0	0	0	0	0	
(neurotransmitter, $G_{i/o}$)	100%	17%	0%	67%	17%	0%	0%	0%	0%	0%	
Serotonin 5-$HT_{2A/B/C}$ Rs	3	0	0	0	0	0	0	0	0	3	5-HT_{2A}(T)/$_B$(T)/$_C$(I)
(neurotransmitter, $G_{q/11}$)	100%	0%	0%	0%	0%	0%	0%	0%	0%	100%	
Histamine H1 R	1	0	0	0	**1**	0	0	0	0	0	
(neurotransmitter, $G_{q/11}$)	100%	0%	0%	0%	**100%**	0%	0%	0%	0%	0%	
Histamine H2 R	1	0	0	**1**	0	0	0	0	0	0	
(neurotransmitter, $G_{q/11}$ > G_s)	100%	0%	0%	**100%**	0%	0%	0%	0%	0%	0%	
Histamine H3/4 Rs	2	0	0	0	0	0	0	1	0	1	H4(R) >
(neurotransmitter, $G_{i/o}$)	100%	0%	0%	0%	0%	0%	0%	50%	0%	50%	H3(S) (G_i)
Melanocortin MC1/2/3/4/5 Rs	5	**5**	0	0	0	0	0	0	0	0	DPxxY-motif
(hormone, G_s)	100%	**100%**	0%	0%	0%	0%	0%	0%	0%	0%	
Acetylcholine (muscarinic) MC1/3/5 Rs	3	**3**	0	0	0	0	0	0	0	0	
(neurotransmitter, $G_{q/11}$)	100%	**100%**	0%	0%	0%	0%	0%	0%	0%	0%	
Acetylcholine (muscarinic) MC2/4 Rs	2	**2**	0	0	0	0	0	0	0	0	
(neurotransmitter, $G_{i/o}$)	100%	**100%**	0%	0%	0%	0%	0%	0%	0%	0%	
Melatonin MTNR1A/B Rs	2	0	0	0	**2**	0	0	0	0	0	NAxxY motif mutant
(hormone, $G_{i/o}$)	100%	0%	0%	0%	**100%**	0%	0%	0%	0%	0%	
Motilin MtlR	1	0	0	0	0	0	**1**	0	0	0	
(peptide, $G_{q/11}$, $G_{12/13}$)	100%	0%	0%	0%	0%	0%	**100%**	0%	0%	0%	
Melanin Conc. Hormone MCH2 R	1	0	0	0	**1**	0	0	0	0	0	
(hormone, $G_{q/11}$)	100%	0%	0%	0%	**100%**	0%	0%	0%	0%	0%	
Melanin Conc. Hormone MCH1 R	1	0	0	0	0	0	0	0	0	**1**	MCH1(T)
(hormone, G_s, $G_{i/o}$, $G_{q/11}$)	100%	0%	0%	0%	0%	0%	0%	0%	0%	**100%**	
Somatostatin SSTR3 R	1	0	0	0	0	0	0	**1**	0	0	R3(R) > R1(N)/2(N)
(hormone, $G_{i/o}$ > $G_{q/11}$)	100%	0%	0%	0%	0%	0%	0%	**100%**	0%	0%	/R4(N)/5(N) (G_i)
Somatostatin SSTR1/2/4/5 Rs	4	0	0	0	**4**	0	0	0	0	0	
(hormone, $G_{i/o}$)	100%	0%	0%	0%	**100%**	0%	0%	0%	0%	0%	
Glycoprotein Hormone FSH R	1	0	0	0	**1**	0	0	0	0	0	FSH(N) >
(hormone, G_s, $G_{i/o}$, $G_{q/11}$)	100%	0%	0%	0%	**100%**	0%	0%	0%	0%	0%	LH(T), TSH(A) (G_s)
Glycoprotein Hormone LHCG/TSH Rs	2	0	0	0	0	0	0	0	0	2	LH(T), TSH(A)
(hormone, G_s > $G_{q/11}$)	100%	0%	0%	0%	0%	0%	0%	0%	0%	100%	
Opioid δ/κ/μOpioid/ORL1 Rs	4	0	0	0	**4**	0	0	0	0	0	
(opioid, $G_{i/o}$)	100%	0%	0%	0%	**100%**	0%	0%	0%	0%	0%	
Chemokine(C) XCR1	1	0	0	0	0	0	**1**	0	0	0	
(chemokine, $G_{i/o}$)	100%	0%	0%	0%	0%	0%	**100%**	0%	0%	0%	
Chemokine(CC) CCR1–10 Rs	10	0	0	0	0	0	6	4	0	0	R2(K)/4–8(K) >
(chemokine, $G_{i/o}$)	100%	0%	0%	0%	0%	0%	60%	40%	0%	0%	R1/3/9/10(R) (G_i)?
Chemokine(CXC) CXCR1–6 Rs	6	0	0	0	1	0	5	0	0	0	R2–6(K) > R1(N) (G_i)
(chemokine, $G_{i/o}$)	100%	0%	0%	0%	17%	0%	83%	0%	0%	0%	
Chemokine(CX3C) CX3CR1	1	0	0	0	0	0	**1**	0	0	0	
(chemokine, $G_{i/o}$)	100%	0%	0%	0%	0%	0%	**100%**	0%	0%	0%	
Atypical Chemokine ACKR1/3/4 Rs	3	0	1	0	1	0	0	0	0	1	ACKR1(Q) >
(chemokine, no signaling/$G_?$)	100%	0%	33%	0%	33%	0%	0%	0%	0%	33%	R3(N),R4(S) ? ($G_?$)
Atypical Chemokine ACKR2	1	0	0	0	0	0	**1**	0	0	0	
(chemokine, arrestin)	100%	0%	0%	0%	0%	0%	**100%**	0%	0%	0%	
total number of Rs	86	total number of subgroups									31
R rate in the largest subgroups: 75/86	87%	rate of conserved subgroups for the 2nd aa of helix 8: 24/31									77%

5-TH, 5-hydroxytrypamine; FSH, follicle-stimulating hormone; LH, luteinizing hormone; TSH, thyroid-stimulating hormone; MC, acetylcholine (muscarinic) receptor.

Table S1. Classification of class A GPCRs by helix 8-2nd residues & subtypes of G proteins (continued).

GPCRs	Helix-8 Second Residue										Predicted Hierarchy,
(signal, G protein subtypes)	all	Glu	Gln	Asp	Asn	His	Lys	Arg	Trp	misc	the 2nd residue, misc.
Angiotensin II R1	1	0	0	0	0	0	**1**	0	0	0	R1(K) > R2(R) (G_i) ?
(hormone, $G_{i/o}$, $G_{q/11}$)	100%	0%	0%	0%	0%	0%	**100%**	0%	0%	0%	
Angiotensin II R2	1	0	0	0	0	0	0	**1**	0	0	
(hormone, $G_{i/o}$)	100%	0%	0%	0%	0%	0%	0%	**100%**	0%	0%	
$GnRH_1$ R	1	0	0	0	0	0	0	0	0	0	no helix 8
(hormone, $G_{q/11} > G_{i/o}$)	100%	0%	0%	0%	0%	0%	0%	0%	0%	0%	
$GnRH_2$ R	1	0	0	0	0	0	0	0	0	0	no NPxxY, no helix 8
(hormone, ?)	100%	0%	0%	0%	0%	0%	0%	0%	0%	0%	
Apelin R	1	0	0	0	0	0	0	**1**	0	0	AGTL1
(peptide, $G_{i/o}$)	100%	0%	0%	0%	0%	0%	0%	**100%**	0%	0%	
Cholecystokinin $CCK_{1/2}$ Rs	2	0	0	0	0	0	0	**2**	0	0	$G_q > G_s$
(peptide, $G_{q/11}$)	100%	0%	0%	0%	0%	0%	0%	**100%**	0%	0%	
Bradykinin 2 R	1	0	0	0	0	0	0	**1**	0	0	R2(R) > R1(L) (G_i)
(peptide chemokine, G_s, $G_{i/o}$, $G_{q/11}$)	100%	0%	0%	0%	0%	0%	0%	**100%**	0%	0%	
Bradykinin 1 R	1	0	0	0	0	0	0	0	0	**1**	R1(L)
(peptide chemokine, $G_{i/o}$, $G_{q/11}$)	100%	0%	0%	0%	0%	0%	0%	0%	0%	**100%**	
Galanin 2 R	1	0	0	0	0	**1**	0	0	0	0	
(peptide hormone, $G_{q/11}$)	100%	0%	0%	0%	0%	**100%**	0%	0%	0%	0%	
Galanin 1/3 Rs	2	0	0	0	1	1	0	0	0	0	R3(H) > R1(N) (G_i)
(peptide hormone, $G_{i/o}$)	100%	0%	0%	0%	50%	50%	0%	0%	0%	0%	
Vassopressin V2 R	1	0	0	0	0	0	0	0	0	**1**	V2(S)
(hormone, G_s)	100%	0%	0%	0%	0%	0%	0%	0%	0%	**100%**	
Vassopressin V1a/b & Oxytocin OXT Rs	3	0	0	0	0	**3**	0	0	0	0	
(hormone, $G_{q/11}$)	100%	0%	0%	0%	0%	**100%**	0%	0%	0%	0%	
Cannabinoid CNR1/2 Rs	2	1	0	1	0	0	0	0	0	0	R1(D) > R2(E) (G_i)
(neurotransmitter/lipid, $G_{i/o} > G_s$)	100%	50%	0%	50%	0%	0%	0%	0%	0%	0%	R2(E) > R1(D) (G_s)
GPER1 (GPR30)	1	0	0	0	0	0	0	0	0	**1**	R1(T)
(hormone, G_s, $G_{i/o}$, $G_{q/11}$)	100%	0%	0%	0%	0%	0%	0%	0%	0%	**100%**	
Adenosine $A_{2A/B}$ Rs	2	1	0	1	0	0	0	0	0	0	A_{2A}(E) > A_{2B}(D) (G_s)
(neurotransmitter, G_s)	100%	50%	0%	50%	0%	0%	0%	0%	0%	0%	
Adenosine $A_{1/3}$ Rs	2	0	0	0	0	0	**2**	0	0	0	
(neurotransmitter, $G_{i/o}$)	100%	0%	0%	0%	0%	0%	**100%**	0%	0%	0%	
Hydrocarboxyl acid $HCA_{1/2/3}$ Rs	3	0	0	0	0	0	0	0	0	**3**	R1/2/3(S), DPxxY-
(organic acid, $G_{i/o}$)	100%	0%	0%	0%	0%	0%	0%	0%	0%	**100%**	motif
Kisspeptin R	1	0	0	0	0	**1**	0	0	0	0	
(neuropeptide, $G_{q/11}$)	100%	0%	0%	0%	0%	**100%**	0%	0%	0%	0%	
Rexaxin/insulin-like family peptide 1/2 Rs	2	0	0	0	0	0	0	0	0	2	R1(P) > R2(F) (G_s)
(peptide hormones, G_s, $G_{i/o}$)	100%	0%	0%	0%	0%	0%	0%	0%	0%	100%	
Rexaxin/insulin-like family peptide 3/4 Rs	2	**2**	0	0	0	0	0	0	0	0	
(peptide hormones, $G_{i/o}$)	100%	**100%**	0%	0%	0%	0%	0%	0%	0%	0%	
Hypocretin (orexin) $OX_{1/2}$ Rs	2	0	0	0	0	0	**2**	0	0	0	
(peptide hormones, G_s, $G_{i/o}$, $G_{q/11}$)	100%	0%	0%	0%	0%	0%	**100%**	0%	0%	0%	
Bombesin BRS3/NMB/GRP Rs	3	0	0	0	0	0	0	0	0	**3**	(S, S, S)
(peptide, $G_{q/11}$)	100%	0%	0%	0%	0%	0%	0%	0%	0%	**100%**	
Endothelin A R	1	0	0	0	0	0	**1**	0	0	0	
(peptide, $G_{q/11}$)	100%	0%	0%	0%	0%	0%	**100%**	0%	0%	0%	
Endothelin B R	1	0	0	0	0	0	0	**1**	0	0	
(peptide, G_s)	100%	0%	0%	0%	0%	0%	0%	**100%**	0%	0%	
Neurotensin NTS_1 R	1	0	0	0	**1**	0	0	0	0	0	R1(N) > R2(S) (G_q)
(neuropeptide, $G_{q/11} > G_s/G_{i/o}$)	100%	0%	0%	0%	**100%**	0%	0%	0%	0%	0%	
Neurotensin NTS_2 R	1	0	0	0	0	0	0	0	0	**1**	R2(S)
(neuropeptide, $G_{q/11}$)	100%	0%	0%	0%	0%	0%	0%	0%	0%	**100%**	
Neuromedin U NMU1/2 Rs	2	0	0	0	0	0	0	**2**	0	0	
(neuropeptide, $G_{q/11}$)	100%	0%	0%	0%	0%	0%	0%	**100%**	0%	0%	
Neuropeptide W/B $NPBW_{1/2}$ Rs	2	0	0	0	1	0	0	0	0	1	R1(N) > R2(S) (G_i)
(neuropeptide, $G_{i/o}$)	100%	0%	0%	0%	50%	0%	0%	0%	0%	50%	
Neuropeptides FF NPFF1/2 Rs	2	0	0	0	**2**	0	0	0	0	0	
(neuropeptide, $G_{i/o}$)	100%	0%	0%	0%	**100%**	0%	0%	0%	0%	0%	
Neuropeptides S NPS R	1	0	0	0	0	0	0	0	0	**1**	R(S)
(neuropeptide, $G_{q/11} > G_s$)	100%	0%	0%	0%	0%	0%	0%	0%	0%	**100%**	
Neuropeptides Y 2/4 Rs	2	0	0	0	**2**	0	0	0	0	0	R1(N)/2(N)/4(N) >
(neuropeptide, $G_{i/o} > G_{q/11}$)	100%	0%	0%	0%	**100%**	0%	0%	0%	0%	0%	R5(G) (G_i)
Neuropeptides Y 1/5 Rs	2	0	0	0	1	0	0	0	0	1	R1(N) > R5(G) (G_i)
(neuropeptide, $G_{i/o}$)	100%	0%	0%	0%	50%	0%	0%	0%	0%	50%	
total number of Rs	51	total number of subgroups									32
R rate in the largest subgroups: 43/51	84%	rate of conserved subgroups for the 2nd aa of helix 8: 24/32									75%

GnRH, gonadotropin-releasing hormone; AGTL1, angiotensin II receptor-like 1; GPER1, G protein-coupled estrogen receptor 1; BRS3, bombesin receptor subtype 3; NMBR, neuromedin B receptor; GRPR, gastrin releasing peptide receptor.

Table S1. Classification of class A GPCRs by helix 8-second residues & subtypes of G proteins (continued).

GPCRs	Helix-8 Second Residue										Predicted Hierarchy,
(signal, G protein subtypes)	all	Glu	Gln	Asp	Asn	His	Lys	Arg	Trp	misc	the 2nd residue, misc.
Proteinase-activated PAR 1/2/4 Rs	3	2	0	1	0	0	0	0	0	0	R1/4(E) > R2(D) (G_q),
(peptide, $G_{i/o}$, $G_{q/11}$)	100%	67%	0%	33%	0%	0%	0%	0%	0%	0%	DPxxY-motif
Proteinase-activated PAR3 R	1	0	0	0	0	0	0	0	0	**1**	R(T)
(peptide, ?)	100%	0%	0%	0%	0%	0%	0%	0%	0%	**100%**	
Ghrelin GHSR	1	0	0	0	0	0	**1**	0	0	0	
(peptide hormone, $G_{q/11} > G_{i/o}$)	100%	0%	0%	0%	0%	0%	**100%**	0%	0%	0%	
Bile acid GPBA R	1	0	0	0	0	0	0	**1**	0	0	
(steroid, G_s)	100%	0%	0%	0%	0%	0%	0%	**100%**	0%	0%	
Formyl Peptide L2 R	1	0	0	0	**1**	0	0	0	0	0	L2(N) > 1(D)/L1(D)
(peptide, $G_{i/o}$, $G_{q/11}$)	100%	0%	0%	0%	**100%**	0%	0%	0%	0%	0%	(G_i)
Formyl peptide 1/L1 Rs	2	0	0	**2**	0	0	0	0	0	0	
(peptide, $G_{i/o}$)	100%	0%	0%	**100%**	0%	0%	0%	0%	0%	0%	
Complement Peptide C5AR1	1	0	0	0	0	0	0	0	0	**1**	C5AR1(G) (G_i > Gq)
(peptide, $G_{i/o} > G_{q/11}$)	100%	0%	0%	0%	0%	0%	0%	0%	0%	**100%**	
Complement Peptide C3AR1	1	0	0	**1**	0	0	0	0	0	0	
(peptide, $G_?$)	100%	0%	0%	**100%**	0%	0%	0%	0%	0%	0%	
Complement Peptide C5AR2	1	0	0	0	0	0	0	0	0	**1**	C5AR2(A)
(peptide, Arrestin)	100%	0%	0%	0%	0%	0%	0%	0%	0%	**100%**	
Tachykinin $NK_{1/2}$ Rs	2	0	0	0	0	0	0	**2**	0	0	
(peptide, G_s, $G_{q/11}$)	100%	0%	0%	0%	0%	0%	0%	**100%**	0%	0%	
Tachykinin NK_3 R	1	0	0	0	0	0	0	**1**	0	0	
(peptide, $G_{q/11}$)	100%	0%	0%	0%	0%	0%	0%	**100%**	0%	0%	
Prolactin-releasing peptide R	1	0	0	0	0	0	0	0	0	**1**	R(S)
(peptide, $G_{q/11}$)	100%	0%	0%	0%	0%	0%	0%	0%	0%	**100%**	
QRFP R	1	0	0	0	**1**	0	0	0	0	0	
(peptide, $G_{i/o}$, $G_{q/11}$)	100%	0%	0%	0%	**100%**	0%	0%	0%	0%	0%	
Prokineticin PKR1/2 Rs	2	0	0	0	0	0	0	0	0	**2**	R1/2(T), NTxxF-motif
(peptide, $G_{q/11} > G_s$)	100%	0%	0%	0%	0%	0%	0%	0%	0%	**100%**	
Urotencin-Ⅱ R	1	0	0	0	**1**	0	0	0	0	0	
(peptide, $G_{q/11}$)	100%	0%	0%	0%	**100%**	0%	0%	0%	0%	0%	
Oxoglutamate OXGR1 R	1	0	0	0	**1**	0	0	0	0	0	
(organic acid, $G_{q/11}$)	100%	0%	0%	0%	**100%**	0%	0%	0%	0%	0%	
Succinate R	1	0	0	0	0	**1**	0	0	0	0	
(organic acid, $G_{i/o}$, $G_{q/11}$)	100%	0%	0%	0%	0%	**100%**	0%	0%	0%	0%	
Purinergic P2Y1 R	1	0	0	0	0	0	0	0	0	**1**	R1(T), DPxxY-motif
(nucleotide, $G_{q/11} > G_{i/o}$)	100%	0%	0%	0%	0%	0%	0%	0%	0%	**100%**	
Purinergic P2Y2 R	1	0	0	0	0	0	0	**1**	0	0	
(nucleotide, $G_{q/11} > G_{i/o}/G_{12}$)	100%	0%	0%	0%	0%	0%	0%	**100%**	0%	0%	
Purinergic P2Y4 R	1	0	0	0	0	0	**1**	0	0	0	(D/H)PxxY-motif
(nucleotide, $G_{q/11}$)	100%	0%	0%	0%	0%	0%	**100%**	0%	0%	0%	
Purinergic P2Y6 R	1	0	0	0	0	0	**1**	0	0	0	
(nucleotide, $G_{q/11} > G_{12}$)	100%	0%	0%	0%	0%	0%	**100%**	0%	0%	0%	
Purinergic P2Y12/13/14 Rs	3	0	0	0	0	0	1	0	0	2	R13(K) >
(nucleotide, $G_{i/o}$)	100%	0%	0%	0%	0%	0%	33%	0%	0%	67%	R14(P), R12(S)
Prostaglandin D DR1 R	1	0	0	0	0	0	0	0	0	**1**	R(P), (N/D)PWxF-
(prostanoid, G_s)	100%	0%	0%	0%	0%	0%	0%	0%	0%	**100%**	motif
Prostaglandin D DR2 R	1	0	0	**1**	0	0	0	0	0	0	(N/D)PWxF-motif
(prostanoid, $G_{i/o}$)	100%	0%	0%	**100%**	0%	0%	0%	0%	0%	0%	
Prostaglandin E2 EP2 R	1	0	0	0	0	0	0	0	0	**1**	R(P), DPWxY-motif
(prostanoid, G_s)	100%	0%	0%	0%	0%	0%	0%	0%	0%	**100%**	
Prostaglandin E2 EP4 R	1	0	0	0	0	0	0	0	0	**1**	R(T), DPWxY-motif
(prostanoid, $G_s > G_{i/o}$)	100%	0%	0%	0%	0%	0%	0%	0%	0%	**100%**	
Prostaglandin E2 EP1 R	1	0	0	0	0	0	0	0	0	**1**	R(A), DPWxY-motif
(prostanoid, $G_{q/11}$)	100%	0%	0%	0%	0%	0%	0%	0%	0%	**100%**	
Prostaglandin E2 EP3 R	1	0	0	0	0	0	0	0	0	**1**	R(I), DPWxY-motif
(prostanoid, $G_{i/o}$)	100%	0%	0%	0%	0%	0%	0%	0%	0%	**100%**	
Prostaglandin F FP R	1	0	0	0	0	0	0	0	0	**1**	R(A), DPWxY-motif
(prostanoid, $G_{q/11}$)	100%	0%	0%	0%	0%	0%	0%	0%	0%	**100%**	
Prostaglandin I2 IP R	1	0	0	0	0	0	0	0	0	**1**	R(A), DPWxF-motif
(prostanoid, $G_s > G_{i/o}$)	100%	0%	0%	0%	0%	0%	0%	0%	0%	**100%**	
Thromboxane A2 TP R	1	0	0	0	0	0	0	0	0	**1**	R(A), DPWxY-motif
(prostanoid, $G_{q/11}$)	100%	0%	0%	0%	0%	0%	0%	0%	0%	**100%**	
total number of Rs	38	total number of subgroups									31
R rate in the largest subgroups: 35/38	92%	rate of conserved subgroups for the 2nd aa of helix 8: 29/31									94%

GHSR, growth hormone secretagogue receptor type 1; C3AR1, complement C3a receptor 1; C5AR1, complement C5a receptor 1; QRFP, proglutamated RFamide peptide; PTGDR, prostaglandin D2 receptor; PTGER1/2/3/4, prostaglandin E receptor 1/2/3/4; PTGFR, prostaglandin F receptor; PTGIR, prostaglandin I2 receptor; TBXA2R, thromboxane A2 receptor.

Table S1. Classification of class A GPCRs by helix 8-second residues & subtypes of G proteins (continued).

GPCRs	Helix-8 Second Residue										Predicted Hierarchy,
(signal, G protein subtypes)	all	Glu	Gln	Asp	Asn	His	Lys	Arg	Trp	misc	the 2nd residue, misc.
Oxoeicosanoid R	1	0	0	0	**1**	0	0	0	0	0	DPxxY-motif
(leukotriene, $G_{i/o}$)	100%	0%	0%	0%	**100%**	0%	0%	0%	0%	0%	
Cysteinyl leukotriene 1/2 Rs	2	0	0	0	**2**	0	0	0	0	0	(N/D)PxxY-motif
(leukotriene, $G_{q/11} > G_{i/o}$)	100%	0%	0%	0%	**100%**	0%	0%	0%	0%	0%	
Leukotriene B4 BLT_1 R	1	0	0	**1**	0	0	0	0	0	0	R2(D) > R(G) (G_i),
(leukotriene, $G_{i/o} > G_{q/11}$)	100%	0%	0%	**100%**	0%	0%	0%	0%	0%	0%	NPxxY-motif
Leukotriene B4 BLT_1 R	1	0	0	0	0	0	0	0	0	**1**	R(G), NPxxY-motif
(leukotriene, $G_{i/o}$, $G_{q/11}$)	100%	0%	0%	0%	0%	0%	0%	0%	0%	**100%**	
Chemerin CMKLR1	1	0	0	**1**	0	0	0	0	0	0	
(adipokine, $G_{i/o}$)	100%	0%	0%	**100%**	0%	0%	0%	0%	0%	0%	
Chemerin GPR1	1	0	0	0	0	0	**1**	0	0	0	
(adipokine, ?)	100%	0%	0%	0%	0%	0%	**100%**	0%	0%	0%	
CCRL2 R	1	0	0	0	0	0	0	0	0	**1**	R(T)
(chemokine, $G_?$)	100%	0%	0%	0%	0%	0%	0%	0%	0%	**100%**	
LPAR1/2	2	**2**	0	0	0	0	0	0	0	0	(N/D)PxxY-motif
(lipid signal, $G_{i/o}$, $G_{q/11}$, G_{12})	100%	**100%**	0%	0%	0%	0%	0%	0%	0%	0%	
LPAR3	1	0	0	**1**	0	0	0	0	0	0	
(lipid signal, $G_{i/o}$, $G_{q/11}$)	100%	0%	0%	**100%**	0%	0%	0%	0%	0%	0%	
LPAR4	1	0	0	0	0	0	0	0	0	**1**	R4(S), DPxxY-motif
(lipid signal, G_s, $G_{i/o}$, $G_{q/11}$, G_{12})	100%	0%	0%	0%	0%	0%	0%	0%	0%	**100%**	
LPAR6	1	0	0	0	0	0	0	0	0	**1**	R6(T), DPxxY-motif
(lipid signal, G_s, $G_{i/o}$, G_{12})	100%	0%	0%	0%	0%	0%	0%	0%	0%	**100%**	
LPAR5	1	0	0	0	0	0	0	0	0	**1**	R5(G), DPxxY-motif
(lipid signal, $G_{q/11}$, G_{12})	100%	0%	0%	0%	0%	0%	0%	0%	0%	**100%**	
S1PR1	1	**1**	0	0	0	0	0	0	0	0	
(lipid mediator, $G_{i/o}$)	100%	**100%**	0%	0%	0%	0%	0%	0%	0%	0%	
S1PR3	1	**1**	0	0	0	0	0	0	0	0	
(lipid mediator, $G_{i/o}$, $G_{q/11}$, $G_{12/13}$)	100%	**100%**	0%	0%	0%	0%	0%	0%	0%	0%	
S1PR4/5	2	1	0	1	0	0	0	0	0	0	R4(E) = R5(D) (G_i)
(lipid mediator, $G_{i/o}$, $G_{12/13}$)	100%	50%	0%	50%	0%	0%	0%	0%	0%	0%	
S1PR2	1	0	0	**1**	0	0	0	0	0	0	
(lipid mediator, G_s, $G_{q/11}$, $G_{12/13}$)	100%	0%	0%	**100%**	0%	0%	0%	0%	0%	0%	
Prokineticin PKR1/2 Rs	2	0	0	0	0	0	0	0	0	**2**	R1/2(T), NTxxF-motif,
(protein, $G_{q/11} > G_s$)	100%	0%	0%	0%	0%	0%	0%	0%	0%	**100%**	8th from F
Platelet-activating factor R	1	0	0	0	0	0	**1**	0	0	0	DPxxY-motif
(lipid, $G_{i/o}$, $G_{q/11}$)	100%	0%	0%	0%	0%	0%	**100%**	0%	0%	0%	
Free fatty acid FFAR1/2/4 Rs	3	1	0	0	0	0	0	1	0	1	R1(G)/2(V)/4(E),
(lipid, $G_{q/11}$)	100%	33%	0%	0%	0%	0%	0%	33%	0%	33%	(N/D)PxxY-motif
Free fatty acid FFAR3	1	0	0	0	0	0	0	0	0	**1**	R3(G), (N/D)PxxY-
(lipid, $G_{i/o}$)	100%	0%	0%	0%	0%	0%	0%	0%	0%	**100%**	motif
GPR18 R	1	0	**1**	0	0	0	0	0	0	0	DVxxY-motif
(N-arachidonoylglycine, $G_{i/o}$, $G_{q/11}$)	100%	0%	**100%**	0%	0%	0%	0%	0%	0%	0%	
GPR119 R	1	**1**	0	0	0	0	0	0	0	0	NPxxY-motif
(N-oleoylethanolamide, G_s)	100%	**100%**	0%	0%	0%	0%	0%	0%	0%	0%	
GPR55 R	1	**1**	0	0	0	0	0	0	0	0	DVxxY-motif
(lysophosphatidylinositol, $G_{q/11}$, $G_{12/13}$)	100%	**100%**	0%	0%	0%	0%	0%	0%	0%	0%	
total number of Rs	29	total number of subgroups									23
R rate in the largest subgroups: 26/29	90%	rate of conserved subgroups for the 2nd aa of helix 8: 21/23									91%

CMKLR1, chemerin chemokine-like receptor 1; CCLR2, chemokine (C-C) receptor-like receptor2; LPAR, lysophosphatidic acid receptor.

Human GPCRs			Human GPCRs			Subclass.G-pr_subtypes
mOR-S6	*TM7-NPxxY*	*helix 8*	*mOR-S6*	*TM7-NPxxY*	*helix 8*	
Rhod(312)	NPVIYIMMNK**Q**FRNCMLTTIC		OPN1SW(309)	NPIIYCFMNK**Q**FQACIMKMVC		Rhod/O.G_t
OPN1MW(328)	NPVIYVFMNR**Q**FRNCILQLFG		OPN1LW(328)	NPVIYVFMNR**Q**FRNCILQLFG		Rhod/O.G_t
OPN4(356)	NPIIYAITHP**K**YRVAIAQHLP		OPN5(312)	NPIIYQVIDY**K**FACCQTGGLK		Rhod/O.G_i/(G_q)
OPN3(315)	NPVIYVFMIR**K**FRRSLLQLLC					Rhodopsin/Opsin.G_i?
β_1AdR(382)	NPIIYC.RSP**D**FRKAFQGLLC		β_2AdR(331)	NPLIYC.RSP**D**FRIAFQELLC		AdR.G_s
β_3AdR(351)	NPLIYC.RSP**D**FRSAFRRLLC					Adrenergic R.G_s
α_{1A}AdR(332)	NPIIYPCSSQ**E**FKKAFQNVLR		α_{1B}AdR(354)	NPIIYPCSSK**E**FKRAFVRTLG		AdR.G_q
α_{1D}AdR(408)	NPLIYPCSSR**E**FRRAFLRLLC					Adrenergic R.G_q
α_{2A}AdR(432)	NPVIYTIFNH**D**FRRAFKKILC		α_{2B}AdR(429)	NPVIYTIFNQ**D**FRRAFRRILC		AdR.G_i
α_{2C} AdR(443)	NPVIYTVFNQ**D**FRRSFKHILF					Adrenergic R.G_i
D1(336)	NPIIYAF.NA**D**FRKAFSTLLG		D5(365)	NPVIYAF.NA**D**FQKVFAQLLG		DopR.G_s
D2(432)	NPIIYTTFNI**E**FRKAFLKILH		D3(389)	NPVIYTTFNI**E**FRKAFLKILS		DopR.G_i
D4(454)	NPVIYTVFNA**E**FRNVFRKALR					Dopamine R.G_i
5-HT_6(326)	NPIIYPLFMR**D**FKRALGRFLP		5-HT_7(390)	NPFIYAFFNR**D**LRTTYRSLLQ		SeroR.G_s
5-HT_4(318)	NPFLYAFLNK**S**FRRAFLIILC					Serotonin R.G_s
5-HT_{2A}(386)	NPLVYTLFNK**T**YRSAFSRYIQ		5-HT_{2C}(374)	NPLVYTLFNK**I**YRRAFSNYLR		SeroR.G_q>G_i
5-HT_{2B}(386)	NPLVYTLFNK**T**FRDAFGRYIT					Serotonin R.G_q
5-HT_{1D}(362)	NPIIYTVFNE**E**FRQAFQKIVP		5-HT_{5A}(344)	NPLIYTAFNK**N**YNSAFKNFFS		SeroR.G_i
5-HT_{1A}(406)	NPVIYAYFNK**D**FQNAFKKIIK		5-HT_{1B}(375)	NPIIYTMSNE**D**FKQAFHKLIR		SeroR.G_i
5-HT_{1E}(350)	NPLLYTSFNE**D**FKLAFKKLIR		5-HT_{1F}(353)	NPLIYTIFNE**D**FKKAFQKLVR		SeroR.G_i
H1(474)	NPLIYPLCNE**N**FKKTFKRILH					Histamine R.G_q
H2(294)	NPILYAALNR**D**FRTGYQQLFC					Histamine R.G_q>G_s
H4(364)	NPLLYPLCHK**R**FQKAFLKIFC		H3(418)	NPVLYPLCHH**S**FRRAFTKLLC		HistR.G_i
MC1(304)	DPLIYAFHSQ**E**LRRTLKEVLT		MC2(282)	DPFIYAFRSP**E**LRDAFKKMIF		MelaR.G_s
MC3(305)	DPLIYAFRSL**E**LRNTFREILC		MC4(308)	DPLIYALRSQ**E**LRKTFKEIIC		MelaR.G_s
MC5(301)	DPLIYAFRSQ**E**MRKTFKEIIC					Melanocortin R.G_s
MTNR1A(301)	NAIIYGLLNQ**N**FRKEYRRIIV		MTNR1B(313)	NAIVYGLLNQ**N**FRREYKRILL		MelatR.G_i
MtlR(361)	NPILYNLISK**K**YRAAAFKLLL					Motilin R.G_q/G_{12}
MCH1(386)	NPFVYIVLCE**T**FRKRLVLSVK					Melanin Concentration Hormone R.G_s/G_i/G_q
MCH2(312)	NPFLYILLSG**N**FQKRLPQIQR					Melanin Concentration Hormone R.G_q
SSTR3(319)	NPILYGFLSY**R**FKQGFRRVLL					Somatstatin R.G_i>G_q
SSTR1(329)	NPILYGFLSD**N**FKRSFQRILC		SSTR2(318)	NPILYAFLSD**N**FKKSFQNVLC		SomaR.G_i
SSTR4(317)	NPILYGFLSD**N**FRRFFQRVLC		SSTR5(310)	NPVLYGFLSD**N**FRQSFQKVLC		SomaR.G_i
FSHR(632)	NPFLYAIFTK**N**FRRDFFILLS					Glycoprotein hormone R.G_s/G_i/G_q
LHCGR(629)	NPFLYAIFTK**T**FQRDFFLLLS		TSHR(684)	NPFLYAIFTK**A**FQRDVFILLS		GlyHR.G_s>G_q
δOpioid(324)	NPVLYAFLDE**N**FKRCFRQLCR		κOpioid(336)	NPILYAFLDE**N**FKRCFRDFCF		OpioR.G_i
μOpioid(344)	NPVLYAFLDE**N**FKRCFREFCI		ORL1(325)	NPILYAFLDE**N**FKACFRKFCC		OpioR.G_i
XCR1(293)	NPVLYVFVGV**K**FRTHLKHVLR					Chemokine(C) R.G_i
CCR2(311)	NPIIYAFVGE**K**FRSLFHIALG		CCR4(310)	NPIIYFFLGE**K**FRKYILQLFK		Chem(C-C)R.G_i
CCR5(303)	NPIIYAFVGE**K**FRNYLLVFFQ		CCR6(322)	NPVLYAFIGQ**K**FRNYFLKILK		Chem(C-C)R.G_i
CCR7(332)	NPFLYAFIGV**K**FRNDLFKLFK		CCR8(306)	NPVIYAFVGE**K**FKKHLSEIFQ		Chem(C-C)R.G_i
CCR1(307)	NPVIYAFVGE**R**FRKYLRQLFH		CCR3(307)	NPVIYAFVGE**R**FRKYLRHFFH		Chem(C-C)R.G_i
CCR9(323)	NPVLYVFVGE**R**FRRDLVKTLK		CCR10(316)	NPVLYAFLGL**R**FRQDLRRLLR		Chem(C-C)R.G_i
CXCR2(320)	NPLIYAFIGQ**K**FRHGLLKILA		CXCR3(324)	NPLLYAFVGV**K**FRERMWMLLL		Chem(CXC)R.G_i
CXCR6(294)	NPVLYAFVSL**K**FRKNFWKLVK		CXCR5(328)	NPMLYTFAGV**K**FRSDLSRLLT		Chem(CXC)R.G_i
CXCR4(308)	NPILYAFLGA**K**FKTSAQHALT		CXCR1(311)	NPIIYAFIGQ**N**FRHGFLKILA		Chem(CXC)R.G_i
CX3CR1(299)	NPLIYAFAGE**K**FRRYLYHLYG					Chemokine(CX3C) R.G_i
ACKR2(318)	SPILYAFSSH**R**FRQYLKAFLA					Atypical chemokine R.Arrestin
ACKR4(309)	NPILYVFMGA**S**FKNYVMKVAR					Atypical Chemokine R.?
ACKR3(321)	NPVLYSFINR**N**YRYELMKAFI		ACKR1(310)	TPLLLALFCH**Q**ATRTLLPSLP		AtChem R.?

Supplementary Fig. S1. Alignment of amino acid sequences of NPxxY motif and helix 8 of class A GPCRs. The 204 human non-olfactory GPCRs are shown. The **bold residue** indicates the 2nd one of helix 8, the position of which in the amino acid sequence is shown in the parentheses. Helix 8 was expected to be formed by hydrophobic residues at the 3rd and more than two of the 7th, 8th, 10th, and 11th positions. The conserved TM7 motif is (N/D)Pxx(Y/F). 5-TH, 5-hydroxytrypamine; ACM, acetylcholine (muscarinic); FSH, follicle-stimulating hormone; LH, luteinizing hormone; TSH, thyroid-stimulating hormone.

Human GPCRs		Human GPCRs		Subclass.G-pr_subtypes
mOR-S6 TM7-NPxxY	*helix 8*	*mOR-S6 TM7-NPxxY*	*helix 8*	
ACM_2(446)	NPACYALCNA**T**FKKTFKHLLM	ACM_4(459)	NPACYALCNA**T**FKKTFRHLLL	AcM R.G_i
ACM_1(424)	NPMCYALCNK**A**FRDTFRLLLL	ACM_3(550)	NPVCYALCNK**T**FRTTFKMLLL	AcM R.G_q
ACM_5(501)	NPICYALCNR**T**FRKTFKMLLL			Acetylcholine(muscarinic) R.G_q
AGT1R(308)	NPLFYGFLGK**K**FKRYFLQLLK			Angiotensin II R.G_q/G_i/G_{12}
AGT2R(324)	NPFLYCFVGN**R**FQQKLRSVFR			AngIIR.$G_{i/o}$
$GnRH_1$	DPLIYGYFSL			Gonadotrophin-releasing hormone R.G_q>G_i
$GnRH_2$	no helix 8			Gonadotrophin-releasing hormone R.?
APLN(315)	NPFLYAFFDP**R**FRQACTSMLC			Apelin R(Angiotensin Receptor-like 1).$G_{i/o}$
CCK1(376)	NPIIYCFMNK**R**FRLGFMATFP	CCK2(396)	NPLVYCFMHR**R**FRQACLETCA	Cholcy.k.R.G_q
BDKRB2(338)	NPLVYVIVGK**R**FRKKSWEVYQ			Bradykinin R.G_s/G_i/G_q
BDKRB1(318)	NPVIYVFVGR**L**FRTKVWELYK			Bradykinin R.G_i/G_q
GALR2(298)	NPIVYALVSK**H**FRKGFRTICA			Galanin R.G_q
GALR3(297)	NPLVYALASR**H**FRARFRRLWP	GALR1(309)	NPIIYAFLSE**N**FRKAYKQVFK	GalR.G_i
V2(331)	NPWIYASFSS**S**VSSELRSLLC			Vasopressin R.G_s
V1a(354)	NPWIYMFFSG**H**LLQDCVQSFP	V1b(344)	NPWIYMGFNS**H**LLPRPLRHLA	VassR.G_q
OXTR(335)	NPWIYMLFTG**H**LFHELVQRFL			Oxytocin R.G_q
CNR1(403)	NPIIYALRSK**D**LRHAFRSMFP	CNR2(305)	NPVIYALRSG**E**IRSSAHHCLA	CannR.G_i>G_s
GPER1(320)	NPLIYSFLGE**T**FRDKLRLYIE			GP Estrogen R1.G_s
A_{2A}(294)	NPFIYAYRIR**E**FRQTFRKIIR	A_{2B}(296)	NPIVYAYRNR**D**FRYTFHKIIS	AdenR.G_s
A_3(288)	NPIVYAYKIK**K**FKETYLLILK	A_1(294)	NPIVYAFRIQ**K**FRVTFLKIWN	AdenR.G_i
HCA1(284)	DPLVYYFSSP**S**FPKFYNKLKI	HCA2(300)	DPVVYYFSSP**S**FPNFFSTLIN	HCA R.G_s
HCA3(300)	DPVVYYFSSP**S**FPNFFSTLIN			Hydrocarboxyl acid R.G_i
KISSR(329)	NPLLYAFLGS**H**FRQAFRRVCP			Kisspeptin R.G_q
RXFP1(687)	NPILYTLTTR**P**FKEMIHRFWY	RXFP2(697)	NPILYTLTTN**F**FKDKLKQLLH	RelaxR.G_s/G_i
RXFP3(395)	NPVLYCLVRR**E**FRKALKSLLW	RXFP4(315)	NPVLYCLLRR**E**PRQALAGTFR	RelaxR.G_i
OX1(364)	NPIIYNFLSG**K**FREQFKAAFS	OX2(370)	NPIIYNFLSG**K**FREEFKAAFS	OrexnR.G_s/G_i/G_q
BRS3(336)	NPFALYWLSK**S**FQKHFKAQLF	NMBR(330)	NPFALYLLSE**S**FRRHFNSQLC	BombR.G_q
GRPR(328)	NPFALYLLSK**S**FRKQFNTQLL			Gastrin releasing peptide R.G_q
EDNRA(375)	NPIALYFVSK**K**FKNCFQSCLC			Endthelin R.G_q
EDNRB(392)	NPIALYLVSK**R**FKNCFKSCLC			Endthelin R.G_s/G_i/G_q
NMUR1(362)	NPVLYSLMSS**R**FRETFQEALC	NMUR2(332)	NPIIYNLLSR**R**FQAAFQNVIS	NeurM U R.G_q
NTS1(370)	NPILYNLVSA**N**FRHIFLATLA			Neurotensin R.G_q>G_s/G_i
NTS2(364)	TPLLYNAVSS**S**FRKLFLEAVS			Neurotensin R.G_q
NPBW2(322)	NPFLYAFLDD**N**FRKNFRSILR	NPBW1(313)	NPFLYAFLDA**S**FRRNLRQLIT	NeurP W/B R.G_i
NPFF1(335)	NPIIYGYFNE**N**FRRGFQAAFR	NPFF2(441)	NPIIYGFFNE**N**FRRGFQEAFQ	NeurP FF R.G_i
NPS(336)	NPLIYCVFSSSI**S**FPCRVIRL			Neuropeptides S R.G_q>G_s
NPY2(332)	NPLLYGWMNS**N**YRKAFLSAFR	NPY4(328)	NPFIYGFLNT**N**FKKEIKALVL	NeP Y R.G_i>G_q
NPY1(326)	NPIFYGFLNK**N**FQRDLQFFFN	NPY5(431)	NPILYGFLNN**G**IKADLVSLIH	NeP Y R.G_i
PAR1(377)	DPLIYYYASS**E**CQRYVYSILC	PAR2(350)	DPFVYYFVSH**D**FRDHAKNALL	PA R.G_i/G_q
PAR4(346)	DPFIYYYVSA**E**FRDKVRAGLF			Proteinase-activated R.G_i/G_q
PAR3(364)	DPFLYFLMSK**T**RNHSTAYLTK	no helix 8		Proteinase-activated R.?
GHSR(329)	NPILYNIMSK**K**YRVAVFRLLG			Ghrelin(Growth hormone secretagogue) R.G_q>G_i
GPBAR(286)	VPVAMGLGDQ**R**YTAPWRAAAQ			Bile acid R.G_s
FPR3(308)	NPILYVFMGR**N**FQERLIRSLP			Formyl peptide R.G_i>G_q
FPR1(307)	NPMLYVFMGQ**D**FRERLIHALP	FPR2(308)	NPMLYVFVGQ**D**FRERLIHSLP	FormP.G_i
C5a1(306)	NPIIYVVAGQ**G**FQGRLRKSLP			Complement Peptide R.$G_{i/o}$>G_q
C3a(441)	NPFLYALLGK**D**FRKKARQSIQ			Complement Peptide R.$G_?$
C5a2(297)	NPMLFLYFGR**A**QLRRSLPAAC			Complement Peptide R.Arrestin
NK1(311)	NPIIYCCLND**R**FRLGFKHAFR	NK2(313)	NPIIYCCLNH**R**FRSGFRLAFR	TachyR.G_s/G_q
NK3(362)	NPIIYCCLNK**R**FRAGFKRAFR			Tachykinin R.G_q

Supplementary Fig. S1. Alignment of amino acid sequences of NPxxY motif and helix 8 of class A GPCRs (continued). NPS may cause a shift in the position of helix 8 by two amino acids. GnRH, gonadotropin-releasing hormone; AGTL1, angiotensin II receptor-like 1; GPER1, G protein-coupled estrogen receptor 1; BRS3, bombesin receptor subtype 3; NMBR, neuromedin B receptor; ENDRA/B, endothelin receptor type A/B; NMUR, neuromedin U receptor; NTSR, neurotensin receptor; FPR, formyl peptide receptor; C3AR1, complement C3a receptor 1; C5AR1, complement C5a receptor 1.

Human GPCRs		Human GPCRs		Subclass.G-pr_subtypes
mOR-S6 TM7 NPxxY	*helix 8*	*mOR-S6 TM7 NPxxY*	*helix 8*	
PrRPR(341)	NPFIYAWLHD**S**FREELRKLLV			Prolactin-releasing peptide R.G_q
QRFP(338)	NPIVYAFMNE**N**FKKNVLSAVC			Pyroglutamylated RFamide peptide R.G_i/G_q
PKR1(349)	NTLCFVTVKND**T**VKYFKKIML	PKR2(340)	NTVCFVTVKNN**T**MKYFKKMML	PKR.G_q>G_s
UR2R(321)	NPFLYTLLTR**N**YRDHLRGRVR			Urotensin R.G_q
OXGR1(308)	NLLLYVVVSD**N**FQQAVCSTVR			Oxoglutamete R.G_q
SucR(301)	NPVFYFLLGD**H**FRDMLMNQLR			Succinate R.G_i/G_q
P2RY1(330)	DPILYFLAGD**T**FRRRLSRATR			Purinergic P2Y R.G_q>G_i
P2RY2(312)	DPVLYFLAGQ**R**LVRFARDAKP			Purinergic P2Y R.G_q>G_i/G_{12}
P2RY4(312)	DPVLYLLTGD**K**YRRQLRQLCG			Purinergic P2Y R.G_q
P2RY6(307)	DPILFYFTQK**K**FRRRPHELLQ			Purinergic P2Y R.G_q>G_s
P2RY8(299)	DPFVYYFASR**E**FQLRLREYLG	P2RY10(311)	DPILYYFMAS**E**FRDQLSRHGS	P2Y R.?
P2RY11(327)	HPLLYMAAVP**S**LGCCCRHCPG			Purinergic P2Y R.G_q>G_s
P2RY12(304)	DPFIYFFLCK**S**FRNSLISMLK	P2RY13(322)	DPLIYIFLCK**K**FTEKLPCMQG	P2Y R.G_i
P2RY14(301)	DPIIYFFLCQ**P**FREILCKKLH			Purinergic P2Y P2Y R.G_i
DR1(329)	DPWIFIIFRS**P**VFRIFFHKIF			Prostagrandin D R.G_s
DR2(310)	NPVLYVLTCP**D**MLRKLRRSLR			Prostagrandin D R.G_i
ER3(352)	DPWVYLLLRK**I**LLRKFCQIRY			Prostagrandin E2 R.G_i>G_q
ER1(357)	DPWVYILLRQ**A**VLRQLLRLLP			Prostagrandin E2 R.G_q>G_i
ER2(321)	DPWVFAILRP**P**VLRLMRSVLC			Prostagrandin E2 R.G_s
ER4(335)	DPWIYILLRK**T**VLSKAIEKIK			Prostagrandin E2 R.G_s>G_i
FR(310)	DPWVYILLRK**A**VLKNLYKLAS			Prostagrandin F R.G_q>G_s
IR(298)	DPWVFILFRK**A**VFQRLKLWVC			Prostagrandin I2 R.G_s>G_i/G_q
TA2R(314)	DPWVYILFRR**A**VLRRLQPRLS			Thromboxane A2 R.G_q
OXER(361)	DPVLYCFSSP**N**FLHQSRALLG			Oxoeicosanoid R.G_i
CYSLTR1(301)	DPLLYFFSGG**N**FRKRLSTFRK	CYSLTR2(321)	NPLLYYFAGE**N**FKDRLKSALR	CysLeR.G_q>G_i
BLTR2(325)	NPVLYVFTAG**D**LLPRAGPRFL			Leukotriene B4 R.$G_{i/o}$>G_q
BLTR1(291)	NPVLYACAGG**G**LLRSAGVGFV			Leukotriene B4 R.G_i/G_q
CMKLR1(322)	NPILYVFMGQ**D**FKKFKVALFS			Chemerine Chemokine-like R1(Anaphylatoxin R).G_i
GPR1(310)	NPILYVLISK**K**FQARFRSSVA			Chemerine R.?
CCRL2(306)	NPLLYAFLDG**T**FSKYLCRCFH			Chemokine(C-C) R.like 2 R.?
LPAR1(317)	NPIIYSYRDK**E**MSATFRQILC	LPAR2(301)	NAAVYSCRDA**E**MRRTFRRLLC	LisAR.G_i/G_q/G_{12}
LPAR3(299)	NPIIYSYKDE**D**MYGTMKKMIC			Lisophosphatidic acid R.G_i/G_q
LPAR4(317)	DPFIYYFTLE**S**FQKSFYINAH			Lisophosphatidic acid R.G_s/G_i/G_q/G_{12}
LPAR6(297)	DPIVYYFTSD**T**IQNSIKMKNW			Lisophosphatidic acid R.G_s/G_i/G_{12}
LPAR5(303)	DPLVYYFSAE**G**FRNTLRGLGT			Lisophosphatidic acid R.G_q/G_{12}
S1PR1(317)	NPIIYTLTNK**E**MRRAFIRIMS			Sphingosine-1-phosphate R.G_i
S1PR2(294)	NPVIYTWRSR**D**LRREVLRPLQ			Sphingosine-1-phosphate R.G_s/G_q/G_{12}
S1PR3(304)	NPVIYTLASK**E**MRRAFFRLVC			Sphingosine-1-phosphate R.G_i/G_q/G_{12}
S1PR4(313)	NPIIYSFRSR**E**VCRAVLSFLC	S1PR5(312)	NPIIYTLTNR**D**LRHALLRLVC	SphPR.G_i/G_{12}
PAF(299)	DPVIYCFLTK**K**FRKHLTEKFY			Platelet-activating factor R.G_i/G_q
FFAR1(284)	NPLVTGYLGR**G**PGLKTVCAAR	FFAR2(279)	DPLLFYFSSS**V**VRRAFGRGLQ	FreeFAR.G_q
FFAR4(345?)	NPILYNMTLCRN**E**WKKIFCCF			Free fatty acid R.G_q
FFAR3(282)	DPFVYYFSSS**G**FQADFHELLR			Free fatty acid R.G_i
GPR18(292)	DVILYYIVSK**Q**FQARVISVML			N-arachidonoylglycine (lipid) R.G_i/G_q
GP119(285)	NPLIYAYWQK**E**VRLQLYHMAL			N-oleoylethanolamide (lipid) R.G_s
GPR55(294)	DVFCYYFVIK**E**FRMNIRAHRP			lysophosphatidylinositol(lipid) R.G_q/G_{12}

Supplementary Fig. S1. Alignment of amino acid sequences of NPxxY motif and helix 8 of class A GPCRs (continued). Some of their helical structures are likely to be unstable. PKR1/2 and FFAR4 may cause a shift in the position of helix 8 by one and two amino acids, respectively. PKR, prokineticin receptor; Suc, succinate; MTNR1A/B, melatonin receptor 1A/B; NPY1/2/4/5R, neuropeptide Y receptor Y1/2/4/5; QRFP, proglutamated RFamide peptide; CMKLR1, chemokine-like receptor 1; CCRL2, chemokine (C-C) receptor-like receptor 2; PTGDR, prostaglandin D2 receptor; PTGER1/2/3/4, prostaglandin E receptor 1/2/3/4; PTGFR, prostaglandin F receptor; PTGIR, prostaglandin I2 receptor; TBXA2R, thromboxane A2 receptor; CYSLTR, cysteinyl leukotriene receptor; LPAR, lysophosphatidic acid receptor.

Table S2. Classification of class B* GPCRs by helix 8-second residues & subtypes of G proteins.

GPCRs	Helix-8 Second Residue										Predicted Hierarchy or
(signal, G protein subtypes)	all	Glu	Gln	Asp	Asn	His	Lys	Arg	Trp	misc	the 2nd residue, misc.
Glucagon GHRH/GIP/GLP1/GCG Rs*	4	**4**	0	0	0	0	0	0	0	0	VAx(L/I)Y-motif
(hormone, G_s)	100%	**100%**	0%	0%	0%	0%	0%	0%	0%	0%	
Glucagon GLP2 R*	1	**1**	0	0	0	0	0	0	0	0	
(hormone, ?)	100%	**100%**	0%	0%	0%	0%	0%	0%	0%	0%	
Secretin SCT R*	1	**1**	0	0	0	0	0	0	0	0	
(hormone, ?)	100%	**100%**	0%	0%	0%	0%	0%	0%	0%	0%	
Corticotropin releasing hormone $CRF_{1/2}$ Rs*	2	**2**	0	0	0	0	0	0	0	0	VSVFY-motif
(hormone, $G_s > G_{q/11}$)	100%	**100%**	0%	0%	0%	0%	0%	0%	0%	0%	
Calcitonin CT R*	1	**1**	0	0	0	0	0	0	0	0	VAxIY-motif
(peptide, G_s)	100%	**100%**	0%	0%	0%	0%	0%	0%	0%	0%	
Caocitonin receptor-like CALRL R*	1	**1**	0	0	0	0	0	0	0	0	VSxIY-motif
(peptide, –)	100%	**100%**	0%	0%	0%	0%	0%	0%	0%	0%	
PAC1 R*	1	**1**	0	0	0	0	0	0	0	0	Vax(L/I)Y-motif
(polypeptide, G_s)	100%	**100%**	0%	0%	0%	0%	0%	0%	0%	0%	
VIP1/2 Rs*	2	**2**	0	0	0	0	0	0	0	0	
(polypeptide, G_s)	100%	**100%**	0%	0%	0%	0%	0%	0%	0%	0%	
Parathyroid hormone R1*	1	**1**	0	0	0	0	0	0	0	0	
(peptide, $G_s > G_{q/11}$)	100%	**100%**	0%	0%	0%	0%	0%	0%	0%	0%	
Parathyroid hormone R2*	1	**1**	0	0	0	0	0	0	0	0	VSxIY-motif
(peptide, G_s, $G_{q/11}$)	100%	**100%**	0%	0%	0%	0%	0%	0%	0%	0%	
total number of Rs	15	total number of subgroups									10
R rate in the largest subgroups: 15/15	100%	rate of conserved subgroups for the 2nd aa of helix 8: 10/10									100%

GHRHR*, growth hormone releasing hormone receptor; GIPR*, gastric inhibitory polypeptide receptor; GLP1/2R*, glucagon-like peptide-1/2 receptor; GCGR*, glucagon receptor; CT*, Calcitonin receptor; CALRL*, calcitonin receptor-like; SCTR*, secretin receptor; PAC1, pituitary adenylate cyclase activating polypeptide1; CRF, corticotropin-releasing factor; VIPR1/2*, vasoactive intestinal polypeptide receptor1/2; PTH1/2*, parathynoid hormone receptor1/2.

Human GPCRs			Human GPCRs			Subclass.G-pr_subtypes
mOR-S6	*TM7-NPxxY*	*helix 8*	*mOR-S6*	*TM7-NPxxY*	*helix 8*	
GHRHR*(382)	VAILYCFLNQ**E**VRTEISRKWH		GIPR*(398)	VSVLYCFINK**E**VQSEIRRGWH		GlucR.G_s
GLP1R*(408)	VAVIYCFVNN**E**VQLEFRKSWE		GCGR*(406)	VAVLYCFLNK**E**VQSELRRRWH		GlucR.G_s
GLP2R*(442)	VAILYCFLNQ**E**VRTEISRKWH					Glucagon-like peptide 2 R.−
CT*(397)	VATIYCFCNN**E**VQTTVKRQWA					Calcitonin R.G_s>G_q
CALRL*(390)	VSTIFCFFNG**E**VQAILRRNWN					Calcitonin gene-related peptide type1 R.−
SCTR*(394)	VAVLYCFLNG**E**VQLEVQKKWQ					Secretin R.−
CRF1*(398)	VSVFYCFLNS**E**VRSAIRKRWH		CRF2*(365)	VSVFYCFFNG**E**VRSAVRKRWH		CRF R.G_s>G_q
PAC1*(406)	VAVLYCFLNG**E**VQAEIKRKWR					PAC1 R.G_s
VIP1*(394)	VAILYCFLNG**E**VQAELRRKWR		VIP2*(381)	VAVLYCFLNS**E**VQCELKRKWR		VIP R.G_s
PTH1*(465)	VAIIYCFCNG**E**VQAEIKKSWS					Parathynoid hormone R.G_s>G_q
PTH2*(419)	VSIIYCYCNG**E**VQAEVKKMWS					Parathynoid hormone R.G_s/G_q

Supplementary Fig. S2. Alignment of amino acid sequences of NPxxY motif and helix 8 of class B* GPCRs. The 15 human GPCRs are shown. The **bold residue** indicates the 2nd one of helix 8, the position of which in the amino acid sequence is shown in the parentheses. Helix 8 was expected to be formed by hydrophobic residues at the 3rd and more than two of the 7th, 8th, 10th, and 11th positions. The conserved TM7 motif is V(A/S)xxY instead of NPxxY. GHRHR*, growth hormone releasing hormone receptor; GIPR*, gastric inhibitory polypeptide receptor; GLP1/2R*, glucagon-like peptide-1/2 receptor; GCGR*, glucagon receptor; CT*, Calcitonin receptor; CALRL*, calcitonin receptor-like; SCTR*, secretin receptor; PAC1*, pituitary adenylate cyclase activating polypeptide1; CRF, corticotropin-releasing factor; VIPR1/2*, vasoactive intestinal polypeptide receptor1/2; PTH1/2*, parathynoid hormone receptor1/2.

Table S3. Classification of class C** GPCRs by helix 8-second residues & subtypes of G proteins.

GPCRs	Helix-8 Second Residue										Predicted Hierarchy or
(signal, G protein subtypes)	all	Glu	Gln	Asp	Asn	His	Lys	Arg	Trp	misc	the 2nd residue, misc.
Calcium CAS R**	1	0	0	0	**1**	0	0	0	0	0	IYIIL-motif, short helix 8 with weak H-core
(ion, $G_{i/o}$, $G_{q/11}$, $G_{12/13}$)	100%	0%	0%	0%	**100%**	0%	0%	0%	0%	0%	
Calcium CAS(GPR6a) R**	1	**1**	0	0	0	0	0	0	0	0	PKCY(M/V/L)-motif
(ion, $G_{q/11}$)	100%	**100%**	0%	0%	0%	0%	0%	0%	0%	0%	
GABA $GABA_B$1/2 Rs**	2	1	0	1	0	0	0	0	0	0	R1(E),R2(D), PK(C/L)(Y/I)(V/T)-motif?
(neurotransmitter, –)	100%	50%	0%	50%	0%	0%	0%	0%	0%	0%	
Metabotropic glutamate $mGlu_{1/5}$ Rs**	2	0	0	0	**2**	0	0	0	0	0	(V/M)YII(I/L)-motif
(amino acid, $G_{q/11} > G_s$)	100%	0%	0%	0%	**100%**	0%	0%	0%	0%	0%	
Metabotropic glutamate $mGlu_{2/3/4/6/7/8}$ Rs**	6	0	0	0	**6**	0	0	0	0	0	(V/L/T)(Y/H)(I/V)I(L/I)-motif
(amino acid, $G_{i/o}$)	100%	0%	0%	0%	**100%**	0%	0%	0%	0%	0%	
Umami tastant TAS1R1 R**	1	0	0	**1**	0	0	0	0	0	0	PKCY(M/V/L)-motif
(tastant, –)	100%	0%	0%	**100%**	0%	0%	0%	0%	0%	0%	
Sweet tastant TAS1R2 R**	1	**1**	0	0	0	0	0	0	0	0	PKCY(M/V/L)-motif
(tastant, –)	100%	**100%**	0%	0%	0%	0%	0%	0%	0%	0%	
Tastant-common TAS1R3 R**	1	0	0	0	0	0	0	0	0	**1**	R3(G), PKCY(M/V/L)-motif
(tastant, –)	100%	0%	0%	0%	0%	0%	0%	0%	0%	**100%**	
total number of Rs	15	total number of subgroups									7
R rate in the largest subgroups: 14/15	93%	rate of conserved subgroups for the 2nd aa of helix 8: 6/7									86%

CAS, calcium-sensing; GABA, γ-amino butyric acid, mGlu, metabotropic glutamate; TAS1, taste 1.

Human GPCRs		Human GPCRs		Subclass.G-pr_subtypes
mOR-S6	*TM7-NPxxY* *helix 8*	*mOR-S6*	*TM7-NPxxY* *helix 8*	
CASR**(867)	IYIILFKPSR**N**TIEEVRCS			Calcium Sensing R.G_{i},G_{q},G_{12}
GPC6a**(842)	PKCYVIICKQ**E**INTKSAFLKM			Calcium Sensing R.G_{q}
$GABA_{B1}$**(865)	PKMRRLITRG**E**WQSEAQDTMK	$GABA_{B2}$**(752)	PKLITLRTNP**D**AATQNRRFQF	GABAR.$G_{i/o}$
$mGlu_{1}$**(845)	MYIIIAKPER**N**VRSAFTTSDV	$mGlu_{5}$**(832)	VYIILAKPER**N**VRSAFTTSTV	mGluR.G_{q}
$mGlu_{2}$**(824)	LHIILFQPQK**N**VVSHRAPTSR	$mGlu_{3}$**(833)	VHIILFQPQK**N**VVTHRLHLNR	mGluR.G_{i}
$mGlu_{4}$**(852)	VYIILFHPEQ**N**VPKRKRSLKA	$mGlu_{8}$**(848)	VYIIIFHPEQ**N**VQKRKRSFKA	mGluR.G_{i}
$mGlu_{6}$**(850)	TYVILFHPEQ**N**VQKRKRSLKA	$mGlu_{7}$**(855)	VYIIIFHPEL**N**VQKRKRSFKA	mGluR.G_{i}
TAS1R1(820)	PKCYVILCRP**D**LNSTEHFQAS	TAS1R2(820)	PKCYMILFYP**E**RNTPAYFNSM	Taste.−
TAS1R3(822)	PKCYLLMRQP**G**LNTPEFFLGG			Taste.−

Supplementary Fig. S3. Alignment of amino acid sequences of NPxxY motif and helix 8 of class C** GPCRs. The 15 human GPCRs are shown. The **bold residue** indicates the 2nd one of helix 8, the position of which in the amino acid sequence is shown in the parentheses. Helix 8 was expected to be formed by hydrophobic residues at the 3rd and more than two of the 7th, 8th, 10th, and 11th positions.Unstable helix 8 or hydrophobic core. The conserved TM7 motif is PKCYxY, VYIIxF or IYIILF instead of NPxxY. CAS, calcium-sensing; GABA, γ-amino butyric acid, mGlu, metabotropic glutamate receptor; TAS1, taste1.

Table S4. Classification of adhesion class GPCRs by helix 8-second residues & subtypes of G proteins.

GPCRs	Helix-8 Second Residue										Predicted Hierarchy or
(signal, G protein subtypes)	all	Glu	Gln	Asp	Asn	His	Lys	Arg	Trp	misc	the 2nd residue, misc.
ADGRB1/2/3 Rs	3	**3**	0	0	0	0	0	0	0	0	FVIxxxHC-motif
(phosphatidylserine/secretin family?, ?)	100%	**100%**	0%	0%	0%	0%	0%	0%	0%	0%	
ADGRA1/2/3 Rs	3	0	0	**3**	0	0	0	0	0	0	(L/A)F(F/V)xxHHC-motif
(glycosaminoglycans/?, ?)	100%	0%	0%	**100%**	0%	0%	0%	0%	0%	0%	
Cadherin EGF LAG G-type CEL1/2/3 Rs	3	2	0	1	0	0	0	0	0	0	R1/2(E), R3(D), PFxxLx(H/F)C-motif
(cadherin, ?)	100%	67%	0%	33%	0%	0%	0%	0%	0%	0%	
ADGRD1/2 Rs	2	**2**	0	0	0	0	0	0	0	0	L(F/Y)IFL(F/V)(H/Y)(C/A)-motif
(?, G_s/?)	100%	**100%**	0%	0%	0%	0%	0%	0%	0%	0%	
ADGRE R1	1	0	**1**	0	0	0	0	0	0	0	xFIFLxHC-motif
(?, $G_{q/11}$)	100%	0%	**100%**	0%	0%	0%	0%	0%	0%	0%	
ADGRE2/3/P4 Rs	3	0	**3**	0	0	0	0	0	0	0	xFIFLVYC, xLLFVVHC-motif
(?, ?)	100%	0%	**100%**	0%	0%	0%	0%	0%	0%	0%	
ADGRE R5	1	0	0	0	0	0	**1**	0	0	0	xFLYLLHC-motif
(?, $G_{12/13}$)	100%	0%	0%	0%	0%	0%	**100%**	0%	0%	0%	
ADGRF1/3/4/5 Rs	4	0	0	0	0	0	**4**	0	0	0	xFILxFG(C/T)-motif
(?, ?)	100%	0%	0%	0%	0%	0%	**100%**	0%	0%	0%	
ADGRF R2	1	0	0	0	0	0	0	0	0	0	no NPxxY, no helix 8
(?, ?)	100%	0%	0%	0%	0%	0%	0%	0%	0%	0%	
ADGRG R1	1	0	**1**	0	0	0	0	0	0	0	FLIFIWYY-motif
(collagen III, $G_{q/11}$, $G_{12/13}$)	100%	0%	**100%**	0%	0%	0%	0%	0%	0%	0%	
ADGRG R2	1	0	0	0	**1**	0	0	0	0	0	FFILLFYC-motif
(?, $G_{q/11}$)	100%	0%	0%	0%	**100%**	0%	0%	0%	0%	0%	
ADGRG R3	1	0	0	0	0	0	0	0	0	**1**	R3(T), WFxILYLP-motif
(beclometasone dipropionate/?, $G_{i/o}$)	100%	0%	0%	0%	0%	0%	0%	0%	0%	**100%**	
ADGRG 4–7 Rs	4	1	0	0	1	0	0	0	0	2	R7(E), R6(N), G4(S), G5(C), x(F/L)xxx(YLP/FHC)
(?, ?)	100%	25%	0%	0%	25%	0%	0%	0%	0%	50%	
ADGRL R1	1	0	0	0	0	0	**1**	0	0	0	xFIFxFHC-motif
(latrotoxin/?, $G_{q/11}$)	100%	0%	0%	0%	0%	0%	**100%**	0%	0%	0%	
ADGRL 2–4 Rs	3	0	0	0	0	0	**3**	0	0	0	xFIFxFHC-motif
(latrotoxin/FLRT3/?, ?)	100%	0%	0%	0%	0%	0%	**100%**	0%	0%	0%	
ADGRV R1	1	0	0	0	0	0	0	0	0	**1**	R(P), >6000 long sequence
(?, ?)	100%	0%	0%	0%	0%	0%	0%	0%	0%	**100%**	
total number of Rs	33	total number of subgroups									16
R rate in the largest subgroups: 28/33	85%	rate of conserved subgroups for the 2nd aa of helix 8: 13/16									81%

Subtypes of target G proteins were obtained from http://www.guidetopharmacology.org. ADGRA/D/E/F/G/L/V, adhesion G protein-coupled receptor A/D/E/F/G/L/V; ADGRB1/2/3, adhesion G protein-coupled receptor brain-specific angiogenesis inhibitor 1/2/3; CELR, cadherin EGF LAG seven-pass G-type receptors.

Human GPCRs			Human GPCRs		Subclass.G-pr_subtypes
mOR-S6	*TM7-NPxxY*	*helix 8*	*mOR-S6*	*TM7-NPxxY*	*helix 8*	
ADGRB1(1190)	FVIVMVHCILRR**E**VQDAVKCRVV		ADGRB2(1208)	FVITAVHCFLRR**E**VQDVVKCQMG		BraAR.G_i?
ADGRB3(1163)	FVIVMVHCILRR**E**VQDAFRCRLR					Brain-specific angiogenesis inhibitor.G_i?
ADGRA1(312)	LFVLIHHCAKRE**D**VWQCWWACCP		ADGRA2(1075)	LFVFTHHCARRR**D**VRASWRACCP		AdGRA.??
ADGRA3(1062)	AFFVVHHCVNRE**D**VRLAWIMTCC					Adhesion GPCR A R.??
CELR1(2707)	PFVLLFHCVLNQ**E**VRKHLKGVLG		CELR2(2615)	PFIFLSYVVLSK**E**VRKALKLACS		CELR.??
CELR3(2777)	LAVLLLFCVLNA**D**ARAAWMPACL					Cadherin EGF LAG seven-pass G-type R.??
ADGRD1(812)	LFIFLFHCLLNS**E**VRAAFKHKTK		ADGRD2(907)	LYIFLVYAACNE**E**VRSALQRMAE		AdGRD.G_s
ADGRE1(850)	AFIFLIHCLLNG**Q**VREEYKWITG					Adhesion GPCR E.G_q
ADGRE2(850)	VFIFLVYCLLSQ**Q**VREQYGKWSK		ADGRE3(604)	FFIFLVYCILSQ**Q**VQKQYQKWFR		AdGRE.G_q/?
ADGRE4P(441)	VLLFVVHCILNR**Q**VRLIILSVIS					Putative Adhesion GPCR P.??
ADGRE5(792)	AFLYLLHCLLNK**K**VREEYRKWAC					Adhesion GPCR E R.G_{12}
ADGRF1(844)	FFILCFGILLDS**K**LQLLFNSKSA		ADGRF2	no NPxxY, no helix 8		AdGRF.??
ADGRF3(1026)	VFILLFGCLMDR**K**IQEALRKRFC		ADGRF4(658)	FFILLFGTIMDH**K**IRDALRMRMS		AdGRF.??
ADGRF5(1272)	LFILLFGCLWDL**K**VQEALLNKFS					Adhesion GPCR F.??
ADGRG1(664)	FLIFIWYWSMRL**Q**ARGGPSPLKS					Adhesion GPCR G.G_q/G_{12}
ADGRG2(885)	FFIFIFYCVAKE**N**VRKQWRRYLC					Adhesion GPCR G.G_q
ADGRG3(532)	WFTILYLPSQST**T**VSSSTARLDQ					Adhesion GPCR G.G_i
ADGRG4(2992)	WFTILYLPSQST**S**VREQWQIHLC		ADGRG7(730)	ILYTVRTKVFQS**E**ASKVLMLLSS		AdGRG.??
ADGRG5(508)	GFFLFLWFCSQR**C**RSEAEAKAQI		ADGRG6(1170)	LFIFIFHCAMKE**N**VQKQWRQHLC		AdGRG.??
ADGRL1(1113)	VFIFVFHCALQK**K**VHKEYSKCLR					Adhesion GPCR L.G_q
ADGRL2(1090)	VFIFIFHCALQK**K**VRKEYGKCFR					Adhesion GPCR L.??
ADGRL3(1107)	MFIFIFHCVLQK**K**VRKEYGKCLR		ADGRL4(670)	MFIFLFLCVLSR**K**IQEEYYRLFK		AdGRL.??
ADGRV1(6162)	MVYFILHNQMCC**P**MKASYTVEMN					Adhesion GPCR V.??

Supplementary Fig. S4. Alignment of amino acid sequences of NPxxY motif and helix 8 of adhesion class GPCRs. The 33 human GPCRs are shown. The **bold residue** indicates the 2nd one of helix 8, the position of which in the amino acid sequence is shown in the parentheses. Helix 8 was expected to be formed by hydrophobic residues at the 3rd and more than two of the 7th, 8th, 10th, and 11th positions. The conserved TM7 motif is Fx(V/I)xxx(H/Y)C, xFIFxF(H/Y)C or LFIFLx(H/Y)C instead of NPxxY. Unstable helix 8 or hydrophobic core. ADGRA/D/E/F/G/L/V, adhesion G protein-coupled receptor A/D/E/F/G/L/V; ADGRB1/2/3, adhesion G protein-coupled receptor brain-specific angiogenesis inhibitor 1/2/3; CELR, cadherin EGF LAG seven-pass G-type receptors.

Table S5. Classification of Frizzled[#] GPCRs by helix 8-second residues & subtypes of G proteins.

GPCRs	helix-8 second residue										
(signal, G protein subtypes)	all	Glu	Gln	Asp	Asn	His	Lys	Arg	Trp	misc	the 2nd residue, NPxxY-motif.
FZD1/6 Rs[#]	2	0	0	0	0	0	**2**	0	0	0	I(T/S)xxFW(I/V)-motif
(Wnt, $G_{i/o}$, $G_{q/11}$)	100%	0%	0%	0%	0%	0%	**100%**	0%	0%	0%	
FZD2/9 Rs[#]	2	0	0	0	0	0	**2**	0	0	0	ITSxFW(I/V)-motif
(Wnt, $G_{i/o}$)	100%	0%	0%	0%	0%	0%	**100%**	0%	0%	0%	
FZD3 R[#]	1	0	0	0	0	0	**1**	0	0	0	IPSxFWV-motif
(Wnt, G_s)	100%	0%	0%	0%	0%	0%	**100%**	0%	0%	0%	
FZD4/10 Rs[#]	2	0	0	0	0	0	**2**	0	0	0	ITSxMWI-motif
(Wnt, $G_{12/13}$)	100%	0%	0%	0%	0%	0%	**100%**	0%	0%	0%	
FZD7 R[#]	1	0	0	0	0	0	**1**	0	0	0	ITTxFWI-motif
(Wnt, G_s, $G_{i/o}$)	100%	0%	0%	0%	0%	0%	**100%**	0%	0%	0%	
FZD5/8 Rs[#]	2	0	0	0	0	0	**2**	0	0	0	ITSxVW(I/V)-motif
(Wnt, ?)	100%	0%	0%	0%	0%	0%	**100%**	0%	0%	0%	
SMO R[#]	1	0	0	0	0	0	0	0	0	**1**	R(A), TGIxMxW-motif
(oxysterol?, G_s)	100%	0%	0%	0%	0%	0%	0%	0%	0%	**100%**	
total number of Rs	11	total number of subgroups									7
R rate in the largest subgroups: 11/11	100%	rate of conserved subgroups for the 2nd aa of helix 8: 7/7									100%

SMO, smoothened.

Human GPCRs		Human GPCRs		Subclass.G-pr_subtypes
mOR-S6 TM7-NPxxY	*helix 8*	*mOR-S6 TM7-NPxxY*	*helix 8*	
FZD1#(625)	ITSGFWIWSG**K**TLNSWRKFYT	FZD6#(498)	ISAVFWVGSK**K**TCTEWAGFFK	Fzd R.G_i/G_q
FZD2#(543)	ITSGFWIWSG**K**TLHSWRKFYT	FZD9#(532)	ITSGVWVWSS**K**TFQTWQSLCY	Fzd R.G_i/?
FZD3#(502)	IPSVFWVGSK**K**TCFEWASFFH			Frizzled R.G_s
FZD4#(499)	ITSGMWIWSA**K**TLHTWQKCSN	FZD10#(526)	ITSGMWIWTS**K**TLQSWQQVCS	Fzd R.G_{12}
FZD7#(552)	ITTGFWIWSG**K**TLQSWRRFYH			Frizzled R.G_s/G_i
FZD5#(525)	ITSGVWIWSG**K**TVESWRRFTS	FZD8#(608)	ITSGVWVWSG**K**TLESWSLCTR	Fzd R.?
SMO#(540)	TGIAMTWWTK**A**TLLIWRRTWC			Smoothened R.G_i/G_{12}

Supplementary Fig. S5. Alignment of amino acid sequences of NPxxY motif and helix 8 of Frizzled# GPCRs. The 11 human GPCRs are shown. The **bold residue** indicates the 2nd one of helix 8, the position of which in the amino acid sequence is shown in the parentheses. Helix 8 was expected to be formed by hydrophobic residues at the 3rd and more than two of the 7th, 8th, 10th, and 11th positions. Unstable helix 8 or hydrophobic core. The conserved TM7 motif is ITSxFW(I/V) or TGIAMxW instead of NPxxY. SMO, smoothened.

GPCRs			GPCRs		Subclass.G-pr_subtypes
mOR-S6	*TM7_NPxxY*	*helix 8*	*mOR-S6*	*TM7_NPxxY*	*helix 8*	
VN1R1(331)	SPFVLIMSDT**H**ISQFCFACRT		VN1R3(291)	SPFVLMSRHP**R**IPRLGSACCG	VN1R.G$_i$
VN1R2(376)	SPFVLMCRDP**S**RSRLCSICCR		*m*Vmn1r224(280)	ITAIISICFP**T**LGPFVMNHDS	VN1R.G$_i$
VN1R4(291)	SPFVLMSCDP**S**VYRFCFAWKR		*m*Vmn1r237(−)	SPFLVISS	VN1R.G$_i$
VN1R5(326)	SPLMLIYADN**Q**IFKTLQMLWF				Vomeronasal 1 R.G$_i$
GPR107(545)	TLVFFVLTGY**K**FRPASDNPYL		GPR137(296)	LPTTLLVGFF**R**VHRPPQDLST	??.?
GPR143(317)	SLAFYGWTGC**S**LGFQSPRKEI		GPR157(285)	NCIMFVLCTR**A**VRTRLFSLCC	??.?
*m*Vmn1r1	SPLVLISTEQ**R**MINCLKNTQG		*m*Vmn1r4	TPLVQISSDN**R**IINRLKNLQS	VN1R.G$_i$
*m*Vmn1r5	TPLVQFSSDN**R**IIIMLKNLQS		*m*Vmn1r6	TPLIQVSFDN**R**IIIMLKNLQS	VN1R.G$_i$
*m*Vmn1r7	TPLVQISSDN**R**IINMLKNIQS		*m*Vmn1r8	TPLVQISSDN**R**IINMLKNIQS	VN1R.G$_i$
*m*Vmn1r9	TPLVQISSDN**R**IIIMLKNLQS		*m*Vmn1r10	TPLVQISSDN**R**IIIMLKNLQS	VN1R.G$_i$
*m*Vmn1r11	TPLVQISSDN**R**IINRLKNLQS		*m*Vmn1r12	TPLVQISSDK**R**ITRMVKNLQS	VN1R.G$_i$
*m*Vmn1r13	TPLLQISSDK**R**VINVMKTLQS		*m*Vmn1r14	TPFVQISSDT**R**VIRVVKNWHS	VN1R.G$_i$
*m*Vmn1r15	TPLVQISSDK**R**IINVLKNLQS		*m*Vmn1r16	TPLVQISSEN**R**IITMLKNRQS	VN1R.G$_i$
*m*Vmn1r17	TPLVQISSDN**R**IIVMLKNMHS		*m*Vmn1r18	SPLVQITSDK**R**IISILKNVHS	VN1R.G$_i$
*m*Vmn1r19	APLVQISSDK**R**IIHILIHILK		*m*Vmn1r20	SPLVQISSDN**R**IIIMVKNMYS	VN1R.G$_i$
*m*Vmn1r21	TPVVQISSDK**R**IINVLKNLRS		*m*Vmn1r22	TPLVQISSDN**R**IINVLKNLWL	VN1R.G$_i$
*m*Vmn1r23	TPLVQISSDK**R**IINVLKNLWL		*m*Vmn1r24	TPLVQITSDE**R**IINVLKNLWP	VN1R.G$_i$
*m*Vmn1r25	TPLVQISSDK**R**VINVLKNLQS		*m*Vmn1r26	TPLVQISSDN**R**ILKCHQAFFK	VN1R.G$_i$
*m*Vmn1r27	SPLVQIGSDN**R**IIIMVKNMYS		*m*Vmn1r28	TPLVQITSDK**R**IISILKNMHS	VN1R.G$_i$
*m*Vmn1r29	TPLVQITSDN**R**IIIMLENMQS		*m*Vmn1r30	TPLVQISSDK**R**VINVLKNSQS	VN1R.G$_i$
*m*Vmn1r31	TPLVQISSDN**R**IIIMLKNMHS		*m*Vmn1r32	TPLIQISDN**R**MIITLKNMQK	VN1R.G$_i$
*m*Vmn1r33	TPLIQISSDN**R**INIMIKNMQK		*m*Vmn1r34	TPLVQISSDK**R**IIIQKKCKNY	VN1R.G$_i$
*m*Vmn1r35	TPLVQISSDK**R**ISMMLKNMQK		*m*Vmn1r36	TPLVQISSEK**R**IIIILKSMQK	VN1R.G$_i$
*m*Vmn1r37	TPLVQISSDK**R**IIIILKSMQK		*m*Vmn1r38	TPLIQISSDN**R**ILMILKSMQK	VN1R.G$_i$
*m*Vmn1r39	TPLIQIISDN**R**IITMLKNMQK		*m*Vmn1r40	NPFVFICTEK**H**IIKFWESKCG	VN1R.G$_i$
*m*Vmn1r41	SPFVLICTEK**R**MIKFWGSMFG		*m*Vmn1r42	TSYSIELFIM**H**IYATVSPFVF	VN1R.G$_i$
*m*Vmn1r43	TSYSIELFMI**H**IYATVSPFVF		*m*Vmn1r44	SPFVFICTEK**H**IIKFLRSMCG	VN1R.G$_i$
*m*Vmn1r45	TSYSIHIFVM**H**IYATVSPFVF		*m*Vmn1r46	SPFVFICTEK**H**IIKFFWSLCG	VN1R.G$_i$
*m*Vmn1r47	SPFVFMSTEK**H**LVNFFRSMCE		*m*Vmn1r48	SPFVFISTEK**H**IVNILRG	VN1R.G$_i$
*m*Vmn1r49	SPFVFICTEK**H**IIKFWESIFG		*m*Vmn1r50	SPFVFICNDK**Y**MIKFVTSMCG	VN1R.G$_i$
*m*Vmn1r51	SPFVFMSTEK**H**IVNCLRSV		*m*Vmn1r52	SPLLVLSNEK**R**ITNLLISMYE	VN1R.G$_i$
*m*Vmn1r53	SPFVFICTEK**R**ITNFLRSMCG		*m*Vmn1r54	SPFLILSTEK**Y**IINIFRSTFG	VN1R.G$_i$
*m*Vmn1r55	SPLLLIFRDP**R**GPCSLYFNVG		*m*Vmn1r56	SPLLLIFRDP**R**GHCSLLFSVG	VN1R.G$_i$
*m*Vmn1r57	SPLLLIFREP**R**GHCSLLFSVG		*m*Vmn1r58	SPFLLIFRDR**K**GHCSLHIIVS	VN1R.G$_i$
*m*Vmn1r59	SPLLLIFRDP**R**GHCSLLFSVG		*m*Vmn1r60	SPLLLIFRDC**K**GHCSVHIMSV	VN1R.G$_i$
*m*Vmn1r61	SPLLLIFRDC**K**GHCSVHIMSV		*m*Vmn1r62	SPLLLIFRDS**K**GHCSLHIMSV	VN1R.G$_i$
*m*Vmn1r63	SPLLLIFRDC**K**GHCSLRIMSV		*m*Vmn1r64	SPFLLICRDP**M**GPCSLLFIVG	VN1R.G$_i$
*m*Vmn1r65	SPLLLIFRDP**M**CPCPVPFIVG		*m*Vmn1r66	FGPFVLINN-Y**S**VRPRLSLVWM	VN1R.G$_i$
*m*Vmn1r67	FGPFVLMNHCT**F**VPRLSLIWMW		*m*Vmn1r68	FAPCVLMSHYS**F**MPRFSLVWTW	VN1R.G$_i$
*m*Vmn1r69	FAPFVLMSHYS**F**MPKLSLTWIR		*m*Vmn1r71	FAPFVLMSHYS**I**VSRLSLVWLR	VN1R.G$_i$
*m*Vmn1r77	VCPFVLITNM**K**FNFSLFLPCF		*m*Vmn1r80	CPFVLISNMK**P**ISNLFLPCFH	VN1R.G$_i$
*m*Vmn1r90	SPLLLIFRDP**S**YPCSLIFNYR		*m*Vmn1r168	SPLLLIFRDP**S**YPCSLIFNYK	VN1R.G$_i$
*m*Vmn1r172	SPLMLIFRGPK**K**ISAKGINTEN		*m*Vmn1r173	SPLMLIFRGPK**K**ISAKGINTEN	VN1R.G$_i$
*m*Vmn1r174	SPLMLIVRGPK**K**ISAKGINTEN		*m*Vmn1r177	SPLLLIFRDP**S**YHCSLIFNYK	VN1R.G$_i$
*m*Vmn1r178	SPLLLIFRDP**K**YPCSVLFNC		*m*Vmn1r181	SPLLLIFRDP**K**GPCSVFFNC	VN1R.G$_i$
*m*Vmn1r184	FGPCVFIKS-Y**S**LMSRCNLAHL		*m*Vmn1r185	FGPCVFMRS-Y**S**LMSRFNLAHL	VN1R.G$_i$
*m*Vmn1r186	SPLLLIFRDS**K**GHCSLHIMSV		*m*Vmn1r187	SPLLLIFRDC**K**GHCSLRIMSV	VN1R.G$_i$

Supplementary Fig. S6. Alignment of amino acid sequences of NPxxY motif and helix 8 of vomeronasal 1 and other GPCRs. The 9 human and 113 murine GPCRs are shown. The **bold residue** indicates the 2nd one of helix 8, the position of which in the amino acid sequence is shown in the parentheses. Helix 8 was expected to be formed by hydrophobic residues at the 3rd and more than two of the 7th, 8th, 10th, and 11th positions. The conserved TM7 motif is SPxxL, TLxxF, LPxxL, SLxxY, NCxxF, or ITxII instead of the NPxxY. Unstable helix 8 or hydrophobic core. VN1R, human vomeronasal 1 receptor; *m*Vmn1r, murine vomeronasal 1 receptor; GPR, human other G protein-coupled receptor.

GPCRs			GPCRs			Subclass.G-pr_subtypes
mOR-S6	*TM7_NPxxY*	*helix 8*	*mOR-S6*	*TM7_NPxxY*	*helix 8*	
*m*Vmn1r188	SPFVLIHRDG**L**LAEQWETLKR		*m*Vmn1r189	SPLVLIHKDG**L**LAECWHAQME		VN1R.G_i
*m*Vmn1r191	SPLVLIHRDG**L**LAGCCSAQ		*m*Vmn1r192	SPYVLISRDF**K**VPNVLHAH		VN1R.G_i
*m*Vmn1r197	SPFMLIHRDE**H**VIKCFHTQ		*m*Vmn1r198	SPYVLISRNV**R**VPNTLHAH		VN1R.G_i
*m*Vmn1r199	SPYVLISRNV**R**VPNTLHAH		*m*Vmn1r200	SPLVLIHRDG**L**LVERWHVQWE		VN1R.G_i
*m*Vmn1r201	SPFVLIHRDG**L**LVDWWHAQME		*m*Vmn1r207	SPLVLIHRDG**L**LVECCHAQCE		VN1R.G_i
*m*Vmn1r208	SPFVLIHRDG**L**LSKFWHAHWE		*m*Vmn1r209	SPLVLIHRDG**L**LVECWHAQWE		VN1R.G_i
*m*Vmn1r210	SPFVLIHRDG**L**LVKFWHAQME		*m*Vmn1r217	SPLVLIHRDG**L**LPACWHAQ		VN1R.G_i
*m*Vmn1r220	SPFVLIQRDG**L**IPVCWHAQ		*m*Vmn1r221	SPLVLIHRDR**I**LVECWYVQME		VN1R.G_i
*m*Vmn1r222	SPFVLIHRDG**L**LTEQWETLKQ		*m*Vmn1r225	ITSIISMCFP**T**LGPFVMSHYS		VN1R.G_i
*m*Vmn1r226	ITAIISMCFP**T**LGPIVISPDF		*m*Vmn1r227	ITAIISMCFP**T**LGPIVIGCDF		VN1R.G_i
*m*Vmn1r228	ITAIIALCFP**T**LGPFVMSHDF		*m*Vmn1r229	ITAIISMGFP**A**IGPFVMSRDF		VN1R.G_i
*m*Vmn1r230	IPFVLMSQSS**P**LSKLCFL		*m*Vmn1r231	ITVIIHLCFP**T**LGPFIVTQDT		VN1R.G_i
*m*Vmn1r232	TTAIISMGFP**T**LGPFVMSRDF		*m*Vmn1r233	CPFLLMSHDS**R**ASSFCLPLKR		VN1R.G_i
*m*Vmn1r335	SPFLLMNHYS**I**ASSHCVPCMR		*m*Vmn1r336	CPFLLMSQDS**R**ISSYLKRNIH		VN1R.G_i
*m*Vmn1rD19	SPLLLTFRDP**K**GPCSVFFNC					Vomeronasal 1 R.G_i

Supplementary Fig. S6. Alignment of amino acid sequences of NPxxY motif and helix 8 of vomeronasal 1 and other GPCRs (continued). The 9 human and 113 murine GPCRs are shown. The **bold residue** indicates the 2nd one of helix 8, the position of which in the amino acid sequence is shown in the parentheses. Helix 8 was expected to be formed by hydrophobic residues at the 3rd and more than two of the 7th, 8th, 10th, and 11th positions. The conserved TM7 motif is SPxxL, TLxxF, LPxxL, SLxxY, NCxxF, or ITxII instead of the NPxxY. Unstable helix 8 or hydrophobic core. VN1R, human vomeronasal 1 receptor; *m*Vmn1r, murine vomeronasal 1 receptor; GPR, human other G protein-coupled receptor.

Human GPCRs		Human GPCRs		Subclass.G-pr_subtypes
mOR-S6 TM7-NPxxY	*helix 8*	*mOR-S6 TM7-NPxxY*	*helix 8*	*agonist*
TA2R1(283)	HSLILILGNP**K**LKQNAKKFLL			Taste2 R.G_{t3} Peptide
TA2R3(290)	HSFILILGNS**K**LKQTFVVMLR			Taste2 R.G_{t3} Chloroquine
TA2R4(286)	HSVLIIITHP**K**LKTTAKKILC			Taste2 R.G_{t3} Colchicine
TA2R5(278)	HSLILIMGIP**R**VKQTCQKILW			Taste2 R.G_{t3} 1,10-Phenanthroline
TA2R7(291)	HSFILILGNN**K**LRHASLKVIW			Taste2 R.G_{t3} Papaverine
TA2R8(291)	HSLILIVLNN**K**LRQTFVRMLT	TA2R41(289)	HPFILIFSNL**K**LRSVFSQLLL	Tas2 R.G_{t3} Chloramphenicol
TA2R9(288)	HSFILIMGNS**K**LREAFLKMLR			Taste2 R.G_{t3} Pirenzepine
TA2R10(283)	HSFILILGNS**K**LKQASLRVLQ			Taste2 R.G_{t3} Strychrine
TA2R13(287)	HSFLLILGNA**K**LRQAFLLVAA	TA2R30(285)	HPFILILGNK**K**LKQIFLSVLR	Tas2 R.G_{t3} Denatorium
TA2R14(286)	HSCVLILGNK**K**LRQASLSVLL			Taste2 R.G_{t3} Picrotoxinin
TA2R16(282)	HSTSLMLSSP**T**LKRILKGKC			Taste2 R.G_{t3} Salicin
TA2R20(285)	HSFILIWGNK**T**LKQTFLSVLW			Taste2 R.G_{t3} Cromolyn
TA2R31(285)	HPFILIWGNK**K**LKQTFLSVLR	TA2R43(285)	HPFILIWGNK**K**LKQTFLSVFW	Tas2 R.G_{t3} Aristolochic acid
TA2R38(303)	HAAILISGNA**K**LRRAVMTILL			Taste2 R.G_{t3} PROP/PTC
TA2R39(317)	HSILLIQDNP**G**LRRAWKRLQL	TA2R50(285)	DSFILIWRTK**K**LKHTFLLILC	Tas2 R.G_{t3} Amarogentin
TA2R40(302)	HSVQLILGNP**G**LRRAWKRFQH			Taste2 R.G_{t3} Humulones
TA2R46(285)	HPFILIWGNK**K**LKQTFLSVLW			Taste2 R.G_{t3} Absinthin
TA2R19(285)	HSFILIMGSR**K**LKQTFLSVLW			Taste2 R.G_{t3} ??
TA2R42(290)	HSLILILGNS**K**LRQTAVRLLW			Taste2 R.G_{t3} ??
TA2R45(285)	HPFILIWGNK**K**LKQTYLSVLW			Taste2 R.G_{t3} ??
TA2R60(302)	HPIILLFSNC**R**LRAVLKSRRS			Taste2 R.G_{t3} ??

Supplementary Fig. S7. Alignment of amino acid sequences of NPxxY motif and helix 8 of taste2 GPCRs. The 25 human GPCRs are shown. The **bold residue** indicates the 2nd one of helix 8, the position of which in the amino acid sequence is shown in the parentheses. Helix 8 was expected to be formed by hydrophobic residues at the 3rd and more than two of the 7th, 8th, 10th, and 11th positions. The conserved TM7 motif is H(S/P)xIL instead of the NPxxY. Unstable helix 8 or hydrophobic core.

Human GPCRs		Human GPCRs		Subclass.G-pr_subtypes
mOR-S6 TM7_NPxxY	*helix 8*	*mOR-S6 TM7_NPxxY*	*helix 8*	
MAS1(290)	NPFIYFFVGSSKKK**R**FKESLK			Orphan MAS1 proto-oncogene R.G_i/G_q
MRGX1(277)	NPIIYFFVG-**S**FRQRQNRQNL	MRGX3(277)	NPIIYFFVG-**S**FRQRQNRQNL	OclsA.G_q
MRGX4(277)	NPIIYFFVG-**S**FRQRQNRQNL			Orphan Mas-related G protein-coupled R X.G_q
MRGX2(284)	NPIIYFFVG-**S**FRKQWRLQQP			Orphan Mas-related G protein-coupled R X.G_i/G_q
MRGRD(282)	NPVIYFLVGS**R**RSHRLPTRSL			Orphan Mas-related G protein-coupled R.G_i/G_q
MRGRE(275)	KPVVYFCLGS**A**QGRRLPLRLV	MRGRF(297)	KPIVYFLAGR**D**KSQRLWEPLR	OclsA.–
MRGRG(256)	KPLIYSGLGR**Q**PGKREPLRSV			Orphan Mas-related G protein-coupled R.–
MAS1L(324)	NPIIYFFVGSLRKK**R**LKESLR			MAS1 proto-ongogene like R.–
GPR42(282)	DPFVYYFSSS**G**FQADFHELLR			lipid? R.??
GPR4(292)	DPILYCLVNE**G**ARSDVAKALH			Orphan clsA.$G_s/G_i/G_q/G_{12}$
GPR6(345)	NPIIYAFRNQ**E**IQRALWLLLC	GPR12(307)	NPVIYAFRNQ**E**IQKALCLICC	OclsA.G_s/G_i
GPR18(292)	DVILYYIVSK**Q**FQARVISVML	GPR68(292)	DPVLYCFVSE**T**THRDLARLRG	OclsA.G_i/G_q
GPR55(294)	DVFCYYFVIK**E**FRMNIRAHRP			Orphan clsA.G_q/G_{12}
GPR17(331)	DPIMYFFVAE**K**FRHALCNLLC			Orphan clsA.$G_i>G_q$
GPR3(303)	NPIIYAFRNQ**D**VQKVLWACCC	GPR26(300)	DPFVYSLLRH**Q**YRKSCKEILN	OclsA.G_s
GPR61(347)	NPFFYGCLNR**Q**IRGELSKQFV	GPR65(297)	DPILYCFVTE**T**GRYDMWNILK	OclsA.G_s
GPR78(300)	DPFTYSLLRR**P**FRQVLAGMVH	GPR101(460)	HPYVYGYMHK**T**IKKEIQDMLK	OclsA.G_s
GPR132(314)	DPIIYVLATD**H**SRQEVSRIHK			Orphan clsA.G_s
GPR20(303)	DPIVYCFVTS**G**FQATVRGLFG	GPR22(373)	HPLLYAFTRQ**K**FQKVLKSKMK	OclsA.G_i
GPR31(288)	NPVVYCFSSP**T**FRSSYRRVFH	GPR35(282)	DAICYYYMAK**E**FQEASALAVA	OclsA.G_i
GPR33(310)	SPTLYLFVGE**N**FKKVFKKSIL	GPR34(333)	DPVMYFLMSS**N**IRKIMCQLLF	OclsA.G_i
GPR37(555)	TPVLLFCLCK**P**FSRAFMECCC	ETBR2(422)	TPVLLLCICR**P**LGQAFLDCCC	OclsA.G_i
GPR84(376)	NPVLYAAMNR**Q**FRQAYGSILK	GPR183(314)	DPFIYFFACK**G**YKRKVMRMLK	OclsA.G_i
GPR21(310)	NCVIYSISNS**V**FQRGLKRLSG	GPR27(344)	NPVVCFLFNR**E**LRDCFRAQFP	OclsA.G_q
GPR39(350)	NPLLYTVSSQ**Q**FRRVFVQVLC	GPR75(381)	NPFIYSRNSA**G**LRRKVLWCLQ	OclsA.G_q
GPR139(291)	NFFLYCFISK**R**FRTMAAATLK			Orphan clsA.G_q
GPR15(308)	NPFIYYIFDS**Y**IRRAIVHCLC	GPR19(336)	KPTLYSIYNA**N**FRRGMKETFC	OclsA.–
GPR25(313)	NPLIYLLLDR**S**FRARALDGAC	GPR32(322)	NPFLYVFVGR**D**FQEKFFQSLT	OclsA.–
GPR45(330)	NPIVYCWRIK**K**FREACIELLP	GPR52(323)	NCVIYSLSNS**V**FRLGLRRLSE	OclsA.–
GPR50(300)	NAVIYGLLNE**N**FRREYWTIFH	GPR62(296)	HPFLYGLLQR**P**VRLALGRLSR	OclsA.–
GPR63(377)	NPLIYYWRIK**K**FHDACLDMMP	GPR82(316)	DPIIFLLLDK**T**FKKTLYNLFT	OclsA.–
GPR83(351)	NPFIYCWLNE**N**FRIELKALLS	GPR85(345)	NPFVCIFSNR**E**LRRCFSTTLL	OclsA.–
GPR87(320)	DPIIYFFMCR**S**FSRRLFKKSN	GPR88(342)	NPLLYTWRNE**E**FRRSVRSVLP	OclsA.–
GPR135(393)	NPVIYAIRNP**N**ISMLLGRNRE	GPR141(289)	DLLLFVFGGS**H**WFKQKIIGLW	OclsA.–
GPR142(420)	NFGLYCFVSK**T**FRATVRQVIH	GPR146(302)	TPLLYRYMNQ**S**FPSKLQRLMK	OclsA.–
GPR148(326)	LTYLYLLRYR**Q**LLGMVRGHLP	GPR149(366)	TPVFVL--SK**R**WTHLPCGCII	OclsA.–
GPR150(408)	NPFVYLFFQA**G**DCRLRRQLRK	GPR151(312)	NPLIFLVMSE**E**FREGLKGVWK	OclsA.–
GPR152(301)	SPFLCLMASA**D**LRTLLRSVLS	GPR153(302)	LPV-FLWACD**R**YRADLKAVRE	OclsA.–
GPR160(298)	IATVYWFNCH**K**LNLKDIGLPL	GPR161(330)	HPLIYGLWNK**T**VRKELLGMCF	OclsA.–
GPR162(329)	LPS-FIWSCE**R**YRADVRTVWE	GPR171(291)	DPILYY-HSK**A**FRSKVTETFA	OclsA.–
GPR173(346)	NPIVCFLLNK**D**LKKCLRTHAP	GPR174(298)	DPVIYYFSTN**E**FRRRLSRQDL	OclsA.–
GPR176(326)	NPVLFLTVNK**S**VRKCLIGTLV	GPR182(326)	TTLALIFIPK**F**WKLGAPPREE	OclsA.–
LGR4(807)	NPVLYVFFNP**K**FKEDWKLLKR			Orphan clsA.–
LGR5(826)	NPLLYILFNP**H**FKEDLVSLRK	LGR6(833)	NPLLYLLFNP**H**FRDDLRRLRP	OclsA.–

Supplementary Fig. S8. Alignment of amino acid sequences of NPxxY motif and helix 8 of orphan class A GPCRs. The 75 human GPCRs and target G proteins (from http://www.guidetopharmacology. org) are shown. The **bold residue** indicates the 2nd one of helix 8, the position of which in the amino acid sequence is shown in the parentheses. Helix 8 was expected to be formed by hydrophobic residues at the 3rd and more than two of the 7th, 8th, 10th, and 11th positions. Some of their helical structures are likely to be unstable. The conserved TM7 motif is (N/D)Pxx(Y/F). MAS1, MAS1 proto-oncogene G protein-coupled receptor; MAS1L, MAS1 proto-oncogene like, G protein-coupled receptor: MRGR, MAS-related G protein-coupled receptor; MRGX, MAS-related G protein-coupled receptor member X; ETBR2, endothelin B receptor-like protein 2; LGR, leucine-rich repeat containing G protein-coupled receptor.

	Human GPCRs		Human GPCRs		Subclass.G-pr_subtypes
	mOR-S6	*TM7_NPxxY* *helix 8*	*mOR-S6*	*TM7_NPxxY* *helix 8*	
	GPR156**(311)	TTINCFIFIP**Q**LKQWKAFEEE	GPR158**(666)	TVTIGLLLIP**K**FSHSSNNPRDD	OclsC.–
	GPR179**(630)	TTTLALIFIP**K**FWKLGAPPRE			Orphan clsC.–
	RAI3**(264)	WVFLLAYVSP**E**FWLLTKQRNP			Orphan retinoic acid-induced protein 3.–
	GPC5B**(299)	IPEIHCTLLP**A**LQENTPNYFD			Orphan clsC.–
	GPC5C**(342)	KGQSMFVENK**A**FSMDEPVAAK	GPC5D**(288)	NACPVTAYQH**S**FQVENQELS	OclsC.–
	GPC6A**(842)	PKCYVIICKQ**E**INTKSAFLKM			Orphan clsC.–

Supplementary Fig. S9. Alignment of amino acid sequences of NPxxY motif and helix 8 of orphan class C** GPCRs. The 8 human GPCRs and target G proteins (from http://www.guidetopharmacology. org) are shown. The **bold residue** indicates the 2nd one of helix 8, the position of which in the amino acid sequence is shown in the parentheses. Helix 8 was expected to be formed by hydrophobic residues at the 3rd and more than two of the 7th, 8th, 10th, and 11th positions. Some of their helical structures are likely to be unstable. The conserved TM7 motif is PKCYxI or TTTxxL instead of the NPxxY. RAI3, retinoic acid-induced protein 3; GPC, G protein-coupled receptor family C member.

Human G proteins

G proteins	α4 G.H4.23	loop G.h4s6.3 G.h4s6.10 G.h4s6.12			S6 G.h4s6.20	α5 C-terminal G.H5.2 G.H5.13
GNAS (343,350,354,356,381)	DEFLR	T	D	RH------	YCYPHFTCAVDTE	DIIQRMHLRQYELL
GNAL (330,337,341,343,368)	DLFLR	T	D	KH------	YCYPHFTCAVDTE	DIIQRMHLKQYELL
GNAI1(305,312,316,318,341)	CQFED	K	T	E--------	IYTHFTCATDTK	DVIIKNNLKDCGLF
GNAI2(306,313,317,319,342)	SKFED	K	T	E--------	IYTHFTCATDTK	DVIIKNNLKDCGLF
GNAI3(305,312,316,318,341)	CQFED	R	T	E--------	IYTHFTCATDTK	DVIIKNNLKECGLY
GNAT1(301,308,312,314,337)	VQFLE	M	V	E--------	IYTHMTCATDTQ	DIIIKENLKDCGLF
GNAT2(305,312,316,318,341)	SQFLD	M	V	E--------	IYSHMTCATDTQ	DIIIKENLKDCGLF
GNAT3(305,312,316,318,341)	NQFLD	L	D	E--------	IYTHMTCATDTQ	DIIIKENLKDCGLF
GNAO (306,313,316,318,341)	AQFES	R	N	E--------	IYCHMTCATDTN	DIIIANNLRGCGLY
GNAZ (306,313,317,319,342)	RQFED	R	T	E--------	IYSHFTCATDTS	DVIIQNNLKYIGLC
GNAQ (311,318,323,325,346)	KMFVD	P	D	DK------	IIYSHFTCATDTE	DTILQLNLKEYNLV
GNA11(311,318,323,325,346)	KMFVD	P	D	DK------	IIYSHFTCA--TE	DTILQLNLKEYNLV
GNA14(307,314,317,319,342)	KLYQD	P	D	EK------	VIYSHFTCATDTD	DTILQLNLREFNLV
GNA15(315,321,328,330,361)	MYTRM	T	E	SKKGARSRR	LFSHYTCATDTQ	DSVLARYLDEINLL
GNA12(335, - ,342,344,368)	CFDRK	-	N	S------	KPLFHHFTTAIDTE	DTILQENLKDIMLQ
GNA13(330, - ,337,339,364)	CFRNK	-	D	QQ-----	KPLYHHFTTAINTE	DTILHDNLKQLMLQ

Supplementary Fig. S10. Alignment of amino acid sequences for the signature parts in C-terminal regions of α4 and α5 of 16 human G proteins. For each G protein, G.H4.23–27 (α4), G.h4s6.3 and G.h4s6.10 (loop), G.h4s6.12–G.H5.2 and G.H5.13–26 (α5) are shown (the common Gα numbering (CGN) system) [12]. The positions of the first residue are shown in the parentheses. Some of them are considered as residues for the selectivity barcode or precoupling (G.h4s6.10, G.h4s6.12, G.H5.2, G.H5.21) [3] or initial, transient and specific interaction (G.H5.21) or non-specific loop–helix interaction (G.h4s6.10) [10] or M3R–$G\alpha_q$ interaction (G.h4s6.12) [13] to GPCRs. The C-terminal 6th and charged residue (red or blue) is predicted for a single residue for the determinant of initial, transient and specific interaction between Gα and GPCRs [10]. In this study, the C-terminal 5th and negatively-charged residue (blue) is predicted for the residue for the initial, transient and specific interaction to helix-8-2nd-Arg/Lys/His GPCRs.

Table S6. Classification of non-olfactory class-A GPCRs by helix 8-second residues and subtypes of G proteins

		Helix-8 second residue									
	all	**Glu**	**Gln**	**Asp**	**Asn**	**His**	**Lys**	**Arg**	**Trp**	**misc**	2^{nd} residue
non-olfactory class A GPCRs	21	**7**	0	**8**	0	0	0	2	0	4	S(2), P(2)
rate (G_s)	100%	**33%**	0%	**38%**	0%	0%	0%	10%	0%	19%	
non-olfactory class A GPCRs	79	9	4	11	**18**	1	**19**	7	0	10	S(6), G(2), P(1), I(1)
rate ($G_{i/o}$)	100%	12%	5%	14%	**23%**	1%	**25%**	9%	0%	10%	
non-olfactory class A GPCRs	36	**7**	0	0	4	**5**	2	**5**	0	**13**	S(5), A(3), T(2), I(1), G(1), V(1)
rate ($G_{q/11}$)	100%	**19%**	0%	0%	11%	**14%**	6%	**14%**	0%	**36%**	
non-olfactory class A GPCRs	2	0	0	0	0	0	0	0	0	2	T(1), A(1)
rate (G_s > $G_{i/o}$)	100%	0%	0%	0%	0%	0%	0%	0%	0%	100%	
non-olfactory class A GPCRs	2	0	0	0	0	0	0	0	0	**2**	T(1), A(1)
rate (G_s > $G_{q/11}$)	100%	0%	0%	0%	0%	0%	0%	0%	0%	**100%**	
non-olfactory class A GPCRs	2	**1**	0	**1**	0	0	0	0	0	0	
rate ($G_{i/o}$ > G_s)	100%	**50%**	0%	**50%**	0%	0%	0%	0%	0%	0%	
non-olfactory class A GPCRs	5	0	0	1	**2**	0	0	1	0	1	G(1)
rate ($G_{i/o}$ > $G_{q/11}$)	100%	0%	0%	20%	**40%**	0%	0%	20%	0%	20%	
non-olfactory class A GPCRs	6	0	0	1	0	0	0	0	0	**5**	T(4), S(1)
rate ($G_{q/11}$ > G_s)	100%	0%	0%	17%	0%	0%	0%	0%	0%	**83%**	
non-olfactory class A GPCRs	6	0	0	0	**2**	0	1	1	0	2	T(1), no-h8(1)
rate ($G_{q/11}$ > $G_{i/o}$)	100%	0%	0%	0%	**33%**	0%	17%	17%	0%	33%	
non-olfactory class A GPCRs	1	0	0	0	**1**	0	0	0	0	0	
rate ($G_{q/11}$ > G_s/$G_{i/o}$)	100%	0%	0%	0%	**100%**	0%	0%	0%	0%	0%	
non-olfactory class A GPCRs	1	0	0	0	0	0	**1**	0	0	0	
rate ($G_{q/11}$ > $G_{12/13}$)	100%	0%	0%	0%	0%	0%	**100%**	0%	0%	0%	
non-olfactory class A GPCRs	2	0	0	0	0	0	0	0	0	**2**	P(1), F(1)
rate (G_s, $G_{i/o}$)	100%	0%	0%	0%	0%	0%	0%	0%	0%	**100%**	
non-olfactory class A GPCRs	2	0	0	0	0	0	0	**2**	0	0	
rate (G_s, $G_{q/11}$)	100%	0%	0%	0%	0%	0%	0%	**100%**	0%	0%	
non-olfactory class A GPCRs	12	**2**	**1**	**2**	**2**	**1**	**2**	0	0	**2**	G(1), L(1)
rate ($G_{i/o}$, $G_{q/11}$)	100%	**17%**	**8%**	**17%**	**17%**	**8%**	**17%**	0%	0%	**17%**	
non-olfactory class A GPCRs	2	**1**	0	**1**	0	0	0	0	0	0	
rate ($G_{i/o}$, $G_{12/13}$)	100%	**50%**	0%	**50%**	0%	0%	0%	0%	0%	0%	
non-olfactory class A GPCRs	3	**1**	0	0	0	0	**1**	0	0	**1**	G(1)
rate ($G_{q/11}$, $G_{12/13}$)	100%	**33%**	0%	0%	0%	0%	**33%**	0%	0%	**33%**	
non-olfactory class A GPCRs	1	0	0	0	0	0	0	0	0	**1**	T(1)
rate (G_s, $G_{i/o}$, $G_{12/13}$)	100%	0%	0%	0%	0%	0%	0%	0%	0%	**100%**	
non-olfactory class A GPCRs	1	0	0	**1**	0	0	0	0	0	0	
rate (G_s, $G_{q/11}$, $G_{12/13}$)	100%	0%	0%	**100%**	0%	0%	0%	0%	0%	0%	
non-olfactory class A GPCRs	3	**3**	0	0	0	0	0	0	0	0	
rate ($G_{i/o}$, $G_{q/11}$, $G_{12/13}$)	100%	**100%**	0%	0%	0%	0%	0%	0%	0%	0%	
non-olfactory class A GPCRs	6	0	0	0	1	0	**2**	1	0	**2**	T(2)
rate (G_s, $G_{i/o}$, $G_{q/11}$)	100%	0%	0%	0%	17%	0%	**33%**	17%	0%	**33%**	
non-olfactory class A GPCRs	1	0	0	0	0	0	0	0	0	**1**	S(1)
rate (G_s, $G_{i/o}$, $G_{q/11}$, $G_{12/13}$)	100%	0%	0%	0%	0%	0%	0%	0%	0%	**100%**	
non-olfactory class A GPCRs	194	**31**	5	**26**	**30**	7	**28**	19	0	48	
rate (misc)	100%	**16%**	3%	**13%**	**15%**	4%	**14%**	10%	0%	25%	
class B* GPCRs	7	**7**	0	0	0	0	0	0	0	0	
rate (G_s)	100%	**100%**	0%	0%	0%	0%	0%	0%	0%	0%	
class B* GPCRs	4	**4**	0	0	0	0	0	0	0	0	
rate (G_s > $G_{q/11}$)	100%	**100%**	0%	0%	0%	0%	0%	0%	0%	0%	
class B* GPCRs	1	**1**	0	0	0	0	0	0	0	0	
rate (G_s, $G_{q/11}$)	100%	**100%**	0%	0%	0%	0%	0%	0%	0%	0%	
class B* GPCRs	12	**12**	0	0	0	0	0	0	0	0	
rate (G_s, G_s > $G_{q/11}$, G_s/$G_{q/11}$)	100%	**100%**	0%	0%	0%	0%	0%	0%	0%	0%	
class C** GPCRs	6	0	0	0	**6**	0	0	0	0	0	
rate ($G_{i/o}$)	100%	0%	0%	0%	**100%**	0%	0%	0%	0%	0%	
class C** GPCRs	5	**2**	0	1	**2**	0	0	0	0	0	
rate ($G_{q/11}$)	100%	**40%**	0%	20%	**40%**	0%	0%	0%	0%	0%	
class C** GPCRs	1	0	0	0	**1**	0	0	0	0	0	
rate ($G_{i/o}$, $G_{q/11}$, $G_{12/13}$)	100%	0%	0%	0%	**100%**	0%	0%	0%	0%	0%	
class C** GPCRs	12	2	0	1	**9**	0	0	0	0	0	
rate (misc)	100%	17%	0%	8%	**75%**	0%	0%	0%	0%	0%	