SUPPLEMENTARY DATA

A **Structures**

G PROTEIN									STRUCTURE				RECEPTOR					OTHER PROTEINS		
Fam.	α	Species	Note	% of Seq	β	Species	γ	Species	Method	PDB	Refined structure	Resolution	UniProt	IUPHAR	Receptor family	Class	Species	Receptor fusion	Antibodies	Other
Gs	αs	Human	-	55	β1	Bovine	γ2	Bovine	cryo-EM	7DHI	-	3.3	ADRB2	β_2	Adrenoceptors	A (Rhodopsin)	Human	-	Nanobody-35	-
Gs	αs	Human	-	49	β1	Bovine	γ2	Bovine	cryo-EM	7DHR	-	3.8	ADRB2	β_2	Adrenoceptors	A (Rhodopsin)	Human	-	Nanobody-35	-
Gs	αs	Human	-	50	β1	Bovine	γ2	Bovine	cryo-EM	7BZ2	-	3.8	ADRB2	β_2	Adrenoceptors	A (Rhodopsin)	Human	-	Nanobody-35	-
Gs	αs	Human	-	59	β1	Human	γ2	Human	cryo-EM	6NI3	-	3.8	ADRB2	β_2	Adrenoceptors	A (Rhodopsin)	Human	-	Nanobody-35	-
Gs	αs	Bovine	-	89	β1	Rat	γ2	Bovine	X-ray	3SN6	-	3.2	ADRB2	β_2	Adrenoceptors	A (Rhodopsin)	Human	T4-Lysozyme	Antibody	-

Showing 1 to 5 of 5 entries (filtered from 216 total entries)

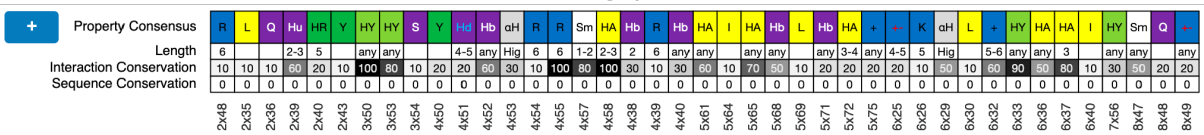
B **Structure models**

MODEL			RECEPTOR					G PROTEIN			TEMPLATE		
Model	Date	Refined	ADRB2	IUPHAR	Rec. family	Class	Species	G protein	G protein	Transducer	Receptor	G protein	PDB
β_2-adrenoceptor - gnai2_human	2020-06-21	No	ADRB2	β_2	Adrenoceptors	A (Rhodopsin)	Human	G(i) subunit alpha-2	GNAI2	secondary	A_1 receptor	G(i) subunit alpha-2	6D9H
β_2-adrenoceptor - gnai1_human	2020-06-21	No	ADRB2	β_2	Adrenoceptors	A (Rhodopsin)	Human	G(i) subunit alpha-1	GNAI1	secondary	μ receptor	G(i) subunit alpha-1	6DDF
β_2-adrenoceptor - gna15_human	2020-06-21	No	ADRB2	β_2	Adrenoceptors	A (Rhodopsin)	Human	subunit alpha-15	GNA15	no evidence	M_1 receptor	subunit alpha-11	6OIJ
β_2-adrenoceptor - gnas2_human	2021-03-15	Yes	ADRB2	β_2	Adrenoceptors	A (Rhodopsin)	Human	G(s) subunit alpha isoforms short	GNAS2	primary	β_2-adrenoceptor	G(s) subunit alpha isoforms short	6NI3
β_2-adrenoceptor - gnai3_human	2020-06-21	No	ADRB2	β_2	Adrenoceptors	A (Rhodopsin)	Human	G(i) subunit alpha-3	GNAI3	secondary	5-HT_{1B} receptor	G(o) subunit alpha	6G79
β_2-adrenoceptor - gnas2_human	2021-03-15	Yes	ADRB2	β_2	Adrenoceptors	A (Rhodopsin)	Human	G(s) subunit alpha isoforms short	GNAS2	primary	β_2-adrenoceptor	G(s) subunit alpha isoforms short	7DHR
β_2-adrenoceptor - gnao_human	2020-06-21	No	ADRB2	β_2	Adrenoceptors	A (Rhodopsin)	Human	G(o) subunit alpha	GNAO	secondary	5-HT_{1B} receptor	G(o) subunit alpha	6G79
β_2-adrenoceptor - gnaz_human	2020-06-21	No	ADRB2	β_2	Adrenoceptors	A (Rhodopsin)	Human	G(z) subunit alpha	GNAZ	secondary	5-HT_{1B} receptor	G(o) subunit alpha	6G79
β_2-adrenoceptor - gnaq_human	2020-06-21	No	ADRB2	β_2	Adrenoceptors	A (Rhodopsin)	Human	G(q) subunit alpha	GNAQ	no evidence	M_1 receptor	subunit alpha-11	6OIJ
β_2-adrenoceptor - gnas2_bovin	2021-03-15	Yes	ADRB2	β_2	Adrenoceptors	A (Rhodopsin)	Human	G(s) subunit alpha isoforms short	GNAS2	primary	β_2-adrenoceptor	G(s) subunit alpha isoforms short	3SN6
β_2-adrenoceptor - gna11_human	2020-06-21	No	ADRB2	β_2	Adrenoceptors	A (Rhodopsin)	Human	subunit alpha-11	GNA11	no evidence	M_1 receptor	subunit alpha-11	6OIJ

Showing 1 to 11 of 11 entries (filtered from 2,197 total entries)

Supplementary Figure 1. Structures and structure models. (**A**) Structure and (**B**) structure model browsers applying a filter to the receptor UniProt name to show β_2-G protein complexes.

A **Interface fingerprint**



B **Interface property consensus conservation in Class**

Toggle **GuideToPharma** / Asuka Inoue / Merged Data | Export to Excel | Export to CSV | Reset All Filters | Show Alignment

Class	Family	IUPHAR	UniProt	Interface Conservation (%)	Gs	Gi / Go	Gq / G11	G12 / G13
	Select values	Select values		11 62	Select value	Select value	Select value	Select value
A	Protein receptors	CCR1	CCR1	62		primary		
A	Peptide receptors	TRH_1 receptor	TRFR	59			primary	
A	Aminergic receptors	D_5 receptor	DRD5	58	primary			
A	Protein receptors	CCR3	CCR3	58		primary		
A	Peptide receptors	NMU2 receptor	NMUR2	56			primary	
A	Peptide receptors	CCK_2 receptor	GASR	55			primary	
A	Protein receptors	CCR4	CCR4	55		primary		
A	Aminergic receptors	5-ht_{1E} receptor	5HT1E	54		primary		
A	Protein receptors	CCR7	CCR7	54		primary		
A	Aminergic receptors	β_3-adrenoceptor	ADRB3	53	primary	secondary		
A	Aminergic receptors	D_4 receptor	DRD4	53		primary		
A	Orphan receptors	*GPR35*	GPR35	53				
A	Aminergic receptors	5-HT_{2C} receptor	5HT2C	53		secondary	primary	
A	Aminergic receptors	β_1-adrenoceptor	ADRB1	53	primary	secondary		
A	Protein receptors	CX_3CR1	CX3C1	53		primary		
A	Protein receptors	XCR1	XCR1	52		primary		
A	Orphan receptors	*TAAR9*	TAAR9	52				
A	Aminergic receptors	5-HT_{1F} receptor	5HT1F	51		primary		
A	Peptide receptors	B_2 receptor	BKRB2	51	primary	primary	primary	

Showing 1 to 269 of 269 entries

Supplementary Figure 2. Interface interactions. **(A)** Interface fingerprint based on residue property consensus of representative class A GPCR-$G_{i/o}$ structures (PDB identifiers: 6OIK, 6D9H, 6K41, 6N4B, 6KPF, 6WWZ, 6LFO, 6OMM, 6OS9 and 6DDE; same as in Fig. 2c). **(B)** Matching of the interface fingerprint listing all other class A GPCRs by decreasing conservation of residue properties along with known G protein couplings.

Mutations to increase G_s binding

1. Mutations to increase Gs binding | 2. Mutations to decrease Gs binding | 3. All residue positions

Mutation design:
To strengthen coupling, mutate receptor positions lacking a conserved positive (+) property by introducing the highest conserved amino acid (AA) of the Gs binding GPCRs having that property.
For negative (-) properties, which contribute to non-binding, instead mutate receptor positions into the highest conserved AA of the Gs binding GPCRs lacking that property.

Residue				Mutant AA	Structure interactions (# receptors)		Mutation data ≥5-fold effect		Gs binders vs non-binders sequence signature				Property conservation Gs binders			Property conservation Gs non-binders			AA conservation Gs binders		AA conservation Gs non-binders		AA conservation GPCR class	
Segment	Target residue number	Generic residue number	Target WT AA	Increase coupling	G protein	Ligand	#Mut.	#Rec.	Score (%)	Property	Length	Matching AAs	Cons. (%)	Property	Length	Cons. (%)	Property	Length	Cons. (%)	AA	Cons. (%)	AA	Cons. (%)	AA
					Min Max	Min Max	Min Max	Min Max	Min Max				Min Max			Min Max			Min Max		Min Max		Min Max	
TM1	43	1x42	I	L	0	4	1	1	31	αH	Hig	A, K, L, M, R	98	HY	any	90	HY	any	38	L	35	V	31	I
TM3	112	3x31	I	F	0	1	4	2	-30	HA	2-3	I, L, V	98	HY	any	88	HY	any	29	L	42	L	37	L
TM7	312	7x38	N	L	0	44	150	19	27	HA	3-4	I, L, M	82	HY	any	58	HY	any	33	L	17	L	20	L
TM5	225	5x64	E	A	9	0	2	1	-27	Hb	any	D, E, H, K,...	62	HY	any	67	Hb	any	22	A	16	K	17	T
TM7	320	7x47	G	A	0	0	1	1	25	HA	any	A, I, L, M, V	78	HY	any	80	HY	any	22	C	47	C	39	C
H8	337	8x55	Q	K	0	0	0	0	25	Hd	5-6	K, R, Y	73	Hb	any	59	Hb	any	24	K	20	R	22	R
TM6	294	6x56	I	V	0	0	2	1	-23	HA	3-4	I, L, M	91	HY	any	89	HY	any	20	I	39	L	36	L
TM4	159	4x51	I	A	0	0	1	1	22	Sm	1-2	A, C, S	91	HY	any	84	HY	any	31	A	32	V	23	V
TM5	220	5x59	S	V	0	0	0	0	-22	Sm	any	A, C, G, S	69	HY	any	60	HY	any	24	V	16	G	15	L
TM4	147	4x39	K	P	3	0	0	0	21	αH	Min	G, P	53	HY	any	50	HY	any	31	P	21	R	19	P
TM5	224	5x63	Q	L	0	0	0	0	21	L		L	69	αH	Hig	51	Hb	any	31	L	26	R	26	R
TM7	317	7x43	V	A	0	0	0	0	21	Sm	any	A, C, G, S	82	HY	any	86	HY	any	24	A	29	L	25	L
H8	338	8x56	E	L	0	0	0	0	21	HA	any	A, I, L, M, V	69	Hb	any	86	Hb	any	13	E	23	R	16	R
TM6	264	6x26	F	H	4	0	0	0	20	Ha	4	E, H, Q	60	Hb	any	50	Hb	any	13	-	25	-	27	-
H8	332	8x50	F	V	0	5	1	1	-20	HY	4-5	F, M, Y	93	HY	any	95	HY	any	56	F	69	F	64	F
TM1	61	1x60	F	N	0	0	0	0	19	Hu	3-4	H, N, Q	84	Hb	any	81	Hb	any	31	N	15	N	14	N
TM3	110	3x29	T	A	0	18	77	10	19	HA	1-2	A, V	53	HY	any	47	HY	any	20	A	17	L	13	G
ICL2	139	34x51	F	L	23	0	2	1	19	L		L	87	HY	any	78	HY	any	49	L	30	L	31	L
ICL2	140	34x52	K	R	4	0	0	0	19	R	6	R	67	Hb	any	66	Hb	any	40	R	21	R	21	R
TM6	266	6x28	L	K	2	0	0	0	19	Hd	4-5	H, K, Q	56	Hb	any	54	Hb	any	13	K	26	R	17	R

Showing 1 to 20 of 65 entries (filtered from 268 total entries) Previous 1 2 3 4 Next

CSV Excel

Mutations to decrease G_s binding

1. Mutations to increase Gs binding | 2. Mutations to decrease Gs binding | 3. All residue positions

Mutation design:
To weaken coupling, mutate receptor positions lacking a conserved negative (-) property by introducing the highest conserved amino acid (AA) of the Gs non-binding GPCRs having that property.
For positive (+) properties, which contribute to binding, instead mutate receptor positions into the highest conserved AA of the Gs non-binding GPCRs lacking that property.

Residue				Mutant AA	Structure interactions (# receptors)		Mutation data ≥5-fold effect		Gs binders vs non-binders sequence signature				Property conservation Gs binders			Property conservation Gs non-binders			AA conservation Gs binders		AA conservation Gs non-binders		AA conservation GPCR class	
Segment	Target residue number	Generic residue number	Target WT AA	Decrease coupling	G protein	Ligand	#Mut.	#Rec.	Score (%)	Property	Length	Matching AAs	Cons. (%)	Property	Length	Cons. (%)	Property	Length	Cons. (%)	AA	Cons. (%)	AA	Cons. (%)	AA
					Min Max	Min Max	Min Max	Min Max	Min Max				Min Max			Min Max			Min Max		Min Max		Min Max	
ICL2	141	34x53	Y	A	11	2	1	1	43	HY	4-5	F, M, Y	82	HY	any	62	Hb	any	62	Y	28	Y	35	Y
TM2	74	2x45	S	N	0	0	0	0	-37	Hb	3	H, N	91	Hb	any	98	Hb	any	56	S	57	N	51	N
TM5	223	5x62	F	I	1	1	0	0	-37	HA	any	A, I, L, M, V	76	HY	any	87	HY	any	29	F	20	I	14	L
TM5	226	5x65	A	L	22	0	10	1	-36	HA	2-3	I, L, V	89	HA	any	93	HY	any	42	A	54	L	46	L
TM1	57	1x56	A	V	0	0	1	1	35	Sm	0-1	A, G	84	HA	any	79	HY	any	51	A	37	V	31	V
TM1	36	1x35	M	L	0	5	4	3	-34	HA	2-3	I, L, V	67	HY	any	83	HY	any	16	L	25	L	27	L
TM5	215	5x54	M	I	0	0	0	0	-34	HA	2-3	I, L, V	84	HA	any	87	HA	any	51	M	39	I	34	I
ICL2	145	34x57	L	R	6	0	0	0	33	HA	any	A, I, L, M, V	51	αH	Hig	61	Hb	any	27	R	40	R	31	R
TM2	73	2x44	T	L	0	0	1	1	-33	αH	Hig	A, K, L, M, R	80	HY	any	81	HY	any	38	V	36	L	33	L
TM4	154	4x46	I	C	0	0	0	0	32	HA	2-3	I, L, V	91	HY	any	78	HY	any	53	I	33	I	30	I
TM3	107	3x26	E	K	0	1	19	4	-32	+	any	H, K, R	80	Hb	any	85	Hb	any	22	K	34	K	34	K
TM3	118	3x37	T	Y	0	15	69	11	-32	HR	4-5	F, H, Y	64	Hb	any	67	Hb	any	27	T	30	Y	26	Y
TM4	172	4x64	H	F	0	2	5	2	-32	HY	any	A, C, F, I, L...	42	-		56	HY	any	42	-	26	-	31	-
TM6	279	6x41	M	V	0	0	2	1	-32	HA	1-2	A, V	87	HY	any	96	HY	any	24	L	49	V	41	V
TM3	109	3x28	W	V	0	12	57	12	31	HR	any	F, H, W, Y	76	HY	any	73	HY	any	22	F	27	V	19	V
TM1	47	1x46	I	G	0	1	3	1	-31	G	0	G	62	Sm	any	76	Sm	any	29	G	60	G	54	G
TM5	229	5x68	Q	R	22	0	0	0	29	Hd	4	H, Q	76	Hb	any	54	Hb	any	24	Q	22	R	17	R
TM6	268	6x30	E	K	2	0	8	4	29	E	4	E	91	Hb	any	80	Hb	any	51	E	22	E	25	E
TM2	71	2x42	F	Y	0	1	0	0	28	F		F	100	HY	any	98	HY	any	67	F	39	F	38	F
TM5	218	5x57	V	C	0	1	0	0	-28	C	2	C	87	HY	any	86	HY	any	20	A	44	C	38	C

Showing 1 to 20 of 131 entries (filtered from 268 total entries) Previous 1 2 3 4 5 6 7 Next

CSV Excel

Supplementary Figure 3. Coupling determinant mutation design. The Coupling determinant mutation design tool (https://gproteindb.org/mutations/gprot_coupling) suggests mutations in a receptor to increase (top) or decrease (bottom) the coupling to a given G protein family (here the β_2-adrenoceptor and G_s family). The determinants suggested for mutagenesis are predicted by tailoring the functionality of a sequence signature tool which identifies distinctly conserved residues within one of two sets of receptors (1) (here G_s- and non-G_s-coupling class A GPCRs).

Supplementary Table 1. Distance and angle rules for defining GPCR – G protein interactions. The values are adapted and extended from (2).

Interaction	Moiety 1	Moiety 2	Max distance (Å)	Angle rule
Hydrogen bond (strong)	Donor	Acceptor	3.5	$\langle \overrightarrow{\text{Donor} - \text{Hydrogen}}, \overrightarrow{\text{Hydrogen} - \text{Acceptor}} \rangle \in [\frac{-\pi}{3}, \frac{\pi}{3}]$
Hydrogen bond (weak)	Donor	Acceptor	4.0	
Ionic	Cation	Anion	4.0	
Hydrophobe	Hydrophobic atom	Hydrophobic atom	4.5	
Aromatic (Face to face)	Ring center	Ring center	4.4	$\langle \overrightarrow{ring\ normal_1}, \text{ring } \overrightarrow{normal_2} \rangle \in [\frac{-\pi}{6}, \frac{\pi}{6}]$
Aromatic (Edge to face)	Ring center	Ring center	5.5	$\langle \overrightarrow{ring\ normal_1}, \text{ring } \overrightarrow{normal_2} \rangle \in [\frac{\pi}{6}, \frac{5\pi}{6}]$
Aromatic (π-cation)	Ring center	Cation	6.6	$\langle \overrightarrow{ring\ normal}, \overrightarrow{\text{ring center} +} \rangle \in [\frac{-\pi}{6}, \frac{\pi}{6}]$
Van-der-Waals (vdW)	vdW Atom	vdW Atom	1.1*atom radii sum	

REFERENCES

1. Kooistra, A.J., Mordalski, S., Pándy-Szekeres, G., Esguerra, M., Mamyrbekov, A., Munk, C., Keserű, G.M. and Gloriam, David E. (2021) GPCRdb in 2021: integrating GPCR sequence, structure and function. *Nucleic Acids Res.*, **49**, D335-D343.
2. Marcou, G. and Rognan, D. (2007) Optimizing fragment and scaffold docking by use of molecular interaction fingerprints. *J. Chem. Inf. Model.*, **47**, 195-207.