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Supplementary information

Autoregulation of GPCR signalling through the third intracellular loop

In the format provided by the authors and unedited

Receptor	Mutation Style	Positions	Insertion	Positions	Ligand Class	pKd,Mut-pKd,WT	Assay	pEC50,Mut-pEC50,WT	Emax,Mut/Emax,WT	Notes	Ref
5HT2B	Insertion	N11-C12	**BRIL**		agonist	0.19					73
5HT2B	Insertion	N11-C14	**BRIL**		agonist	-0.10					73
5HT2B	Insertion	N11-C14	**BRIL**		agonist	0.17					74
5HT7R (mouse)	Directed Mutation	C8	G		agonist	0.00	cAMP	0.68	0.33		75
5HT7R (mouse)	Directed Mutation	C6	S		agonist	-0.13	cAMP		0.22		75
AA2AR	Deletion	N10-C15			agonist	0.40					76
AA2AR	ICL3 replacement	N1-C18	**AA1R**		agonist	-0.18	cAMP	0.13	0.47	+GppNhp condition	77
AA2AR	Directed Mutation	C17-C16	A,A		antagonist	0.06	cAMP	-0.29	0.81		77
AA2AR	ICL3 replacement	C14-C11	**AA1R**		antagonist	-0.03	cAMP	-0.07	0.74		77
AA2AR	ICL3 replacement	C14-C7	**AA1R**		antagonist	0.02	cAMP	0.16	1.05		77
AA2AR	ICL3 replacement	N16-C18	**AA1R**		antagonist	0.09	cAMP	-0.25	0.97		77
AA2AR	Directed Mutation	N1,N3	**AA1R**		antagonist	-0.06	cAMP	0.01	0.80		77
AA2AR	Directed Mutation	N12	N		agonist	0.25	cAMP	-0.49	1.24	+GppNhp condition	77
AA2AR	Directed Mutation	N14	Q		agonist	-0.07	cAMP	-0.72	1.17	+GppNhp condition	77
AA2AR	ICL3 replacement	N1-C2	**AA1R**		agonist	0.36	cAMP	-0.73	0.25	+GppNhp condition	77
AA2AR	Insertion	N10-C15	**T_4L**		agonist						78
AA2AR	Insertion	N10-C15	**T_4L**		agonist	0.48					79
ACM1	Deletion	N10-N22			agonist	0.45	IP3	-0.43	0.53	EC50 value is an average of two given values	80
ACM1	ICL3 replacement	N25-C11	**ADRB1 (Turkey)**	N22-C11	agonist	0.27	IP3		0.70	Derived from Table 2 (- PTX)	81
ACM1	Deletion	C24-C10			agonist	0.26	IP3	0.36	0.63		80
ACM1	Directed Mutation	N5,N7,N12	A,A,K		agonist		IP3	0.14	1.00		80
ACM1	Directed Mutation	C9,C7	A,A		agonist		IP3	-0.32	0.69		80
ACM1	Deletion	N11-C54			agonist	-0.14	IP3	-0.34	0.85		80
ACM1	ICL3 replacement	N(-8)-C3	**ACM2**	N(-8)-C4	antagonist	-0.34	current response		0.01		82
ACM1	Insertion	N10-C14	**T_4L**		agonist	2.45				additional mutations: N2Q,N12Q, N110Q	83
ACM1	Directed Mutation	N2		A	agonist	-0.24	IP3		0.16		84
ACM1	Directed Mutation	N3		A	agonist	-0.16	IP3		0.50		84
ACM1	Deletion	C23-C10					IP3		0.71		85
ACM1	Deletion	N58-C75					IP3		0.87		85
ACM1	Deletion	N56-N65					IP3		1.03		85
ACM1	Deletion	N67-N73					IP3		0.87		85
ACM1	Deletion	N75-C75					IP3		0.95		85
ACM1	Deletion	N46-C58					IP3		0.71		85
ACM1	Deletion	N38-C63					IP3		0.85		85
ACM1	Deletion	N33-C56					IP3		0.95		85

ACM1	Deletion	N11-C54			agonist	-0.23	IP3		0.71		[85]
ACM1	Deletion	N10-C35					IP3		0.89		[85]
ACM1	Deletion	N11-C19			agonist	0.34	IP3		0.87		[85]
ACM1	Deletion	N23-C10			agonist	-0.61	IP3		1.19		[85]
ACM1	Directed Mutation	N(-1),N2,N3		A,A,A			IP3		0.34		[86]
ACM1	Directed Mutation	C8,C6,C2	A,A,A				IP3		0.39		[86]
ACM1	Deletion	N10-C35					IP3		1.40		[87]
ACM1	Deletion	N11-C19					IP3		1.00		[87]
ACM1	Deletion	N23-C10					IP3		1.21		[87]
ACM1	Directed Mutation	C7	A		agonist	-0.10	IP3	-0.72	1.00		[88]
ACM1	Directed Mutation	C6	A		agonist	-0.34	IP3	-1.10	0.46		[88]
ACM1	Directed Mutation	C3	A		agonist	-0.27	IP3	0.00	0.46		[88]
ACM1	Directed Mutation	C6,C3	A,A		agonist	-0.85	IP3	-0.86	0.46		[88]
ACM1	Directed Mutation	C7,C3	A,A		agonist	0.11	IP3	1.52	0.31		[88]
ACM1	Directed Mutation	C7,C6	A,A		agonist	-0.49					[88]
ACM1	Directed Mutation	C7,C6,C3	A,A,A		agonist	-0.23					[88]
ACM1 (mouse)	Insertion	N12-C25	RIR		antagonist	0.01	IP1		0.81		[89]
ACM1 (mouse)	Insertion	N12-C84	RIR		antagonist	0.00	IP1		1.03		[89]
ACM1 (mouse)	Insertion	N12-C67	RIR		antagonist	0.00					[89]
ACM1 (mouse)	Insertion	C69-C25	RIR		antagonist	0.00					[89]
ACM2	ICL3 replacement	N(-7)-C1	**ACM1**	N(-7)-C2	antagonist	0.00					[82]
ACM2	ICL3 replacement	N1-C2	**ACM3**	N1-C3	agonist	-0.88	IP3				[3]
ACM2	ICL3 replacement	N1-N16	**ACM3**	N1-N17	agonist	0.17	IP3				[3]
ACM2	ICL3 replacement	N1-C2	**ACM3**	N1-C3	agonist	-1.13	cAMP inhibition		0.02		[90]
ACM2	ICL3 replacement	N1-N16	**ACM3**	N1-N17	agonist	0.26	cAMP inhibition	-0.65	0.40		[90]
ACM2	ICL3 replacement	N1-C3	**ACM3**		agonist	-0.04	current response		1.74		[91]
ACM2	ICL3 replacement	N1-N21	**ACM3**		agonist	0.13	current response		1.21		[91]
ACM2	ICL3 replacement	N1-N12	**ACM3**		agonist	-0.77	current response		0.44		[91]
ACM2	ICL3 replacement	N13-N21	**ACM3**		agonist	-0.19	current response		1.60		[91]
ACM2	Insertion	N11-C14	**T_4L**		agonist	0.08					[92]
ACM2	Directed Mutation	N3		Y	agonist	0.15	IP3				[93]
ACM2	ICL3 replacement	N1-N11	**ADRB1 (Turkey)**	N1-N11	agonist	0.74			0.70		[94]
ACM2	ICL3 replacement	N1-N15	**ADRB1 (Turkey)**	N1-N16	agonist	0.74			0.70		[94]
ACM2	ICL3 replacement	N1-N25	**ADRB1 (Turkey)**	N1-N26	agonist	0.74	G_o activation		0.91		[94]
ACM2	Directed Mutation	N3	Y		agonist	0.68	IP3				[95]
ACM2	ICL3 replacement	N1-N21	**ACM3**	N1-N21	agonist	0.59	IP3				[95]
ACM2	ICL3 replacement	C29-C(-1)	**ACM3**	C29-C(-1)	agonist	-1.08	IP3				[95]
ACM2	Directed Mutation	C3,C2	A,A		agonist	0.67	IP3				[95]
ACM2	ICL3 replacement	C3-C(-1)	**ACM3**	C3-C(-1)			cAMP Inhibition		0.05		[96]

ACM2	Insertion	C1	A		agonist	0.08	cAMP Inhibition		0.62		97
ACM2	Insertion	C7	A		agonist	-0.08	cAMP Inhibition		0.88		97
ACM3	ICL3 replacement	N1-C3	**ACM2**	N1-C2	antagonist	-0.17	IP3		0.27		3
ACM3	ICL3 replacement	N1-N17	**ACM2**	N1-N16	antagonist	-1.74	IP3		0.17		3
ACM3	ICL3 replacement	N1-C3	**ACM2**	N1-C2	agonist	1.72	IP3		0.05		90
ACM3	ICL3 replacement	N1-N17	**ACM2**	N1-N16	agonist	0.27	IP3		0.01		90
ACM3	ICL3 replacement	N1-C3	**ACM2**		agonist	-0.33	current response		0.07		91
ACM3	ICL3 replacement	N1-N21	**ACM2**		agonist	-0.90	current response		0.16		91
ACM3	ICL3 replacement	N1-N12	**ACM2**		agonist	-0.42	current response		0.17		91
ACM3	ICL3 replacement	N13-N21	**ACM2**		agonist	-0.46	current response		0.67		91
ACM3	ICL3 replacement	N10-N21	**ACM2**		agonist	-0.62	current response		0.54		91
ACM3	ICL3 replacement	N5-N21	**ACM2**		agonist		current response		0.50		91
ACM3	Deletion	N3-N12			agonist	-0.19	current response		0.08		91
ACM3	Deletion	C17-C10			agonist		current response		0.97		91
ACM3	Deletion	C9-C6			agonist		current response		0.26		91
ACM3	Insertion	N9-C12	**T_4L**		agonist	0.77					98
ACM3	Insertion	N2		A	agonist	0.11	IP3	-0.99	1.08	3 and 5 alanine insertions disrupt signaling completely	99
ACM3	Directed Mutation	N3		F	agonist	0.15	IP3	-0.12	1.06	Point mutation to W or A at this site have different effects	93
ACM3	ICL3 replacement	N1-N16	**ACM2**	N1-N16	agonist	0.45	IP3		0.13	S(N3)Y rescues IP3 accumulation	93
ACM3	ICL3 replacement	N1-N16	**ACM2**	N1-N16	agonist	0.01	IP3		0.14		100
ACM3	ICL3 replacement	N1-N8	**ACM2**	N1-N8	agonist	0.60	IP3		0.19		100
ACM3	ICL3 replacement	N9-N19	**ACM2**	N9-N19	agonist	0.68	IP3	-0.51	0.96		100
ACM3	ICL3 replacement	N1-N4	**ACM2**	N1-N4	agonist	0.31	IP3	-0.82	0.27		100
ACM3	ICL3 replacement	N5-N8	**ACM2**	N5-N8	agonist	0.98	IP3	0.06	0.98		100
ACM3	Deletion	N1-N8			agonist	0.40	IP3		0.00		100
ACM3	Directed Mutation	N1	**ACM2**	N1	agonist	0.61	IP3	-0.45	1.22		100
ACM3	Directed Mutation	N2	**ACM2**	N2	agonist	0.45	IP3	-0.26	1.08		100
ACM3	Directed Mutation	N3	**ACM2**	N3	agonist	0.56	IP3	-0.82	0.33		100
ACM3	Directed Mutation	N4	**ACM2**	N4	agonist	0.22	IP3	-0.40	0.97		100
ACM3	Directed Mutation	N3-N7		FMLVL	agonist	0.00	current response		1.14		101
ACM3	Directed Mutation	N8-N12		MIVML	agonist	-0.22	current response		0.31		101
ACM3	Directed Mutation	N8,N10-N12		M,VML	agonist	-1.22	current response		1.24		101
ACM3	Deletion	N3-N7			agonist	0.00					101
ACM3	Directed Mutation	C8-C6,C4		VMN,T	agonist	-0.64	current response		0.22		101
ACM3	Deletion	N13-C13			agonist	-0.73	current response		0.77		101

ACM3	Deletion	N13-C10			agonist	-0.22			0.38		101
ACM3	Deletion	N13-C6			agonist	-0.30	current response		0.20		101
ACM3	Deletion, Directed Mutation	N13-C13,C8-C6,C4		VMN,T	agonist	-0.48	current response		0.14		101
ACM3	Insertion	C1	A		agonist	-0.26	IP3		0.16		97
ACM3	Insertion	C7	A		agonist	0.04	IP3		0.81		97
ACM4	Insertion	N10-C13	**T_4L**		agonist	-0.06					83
ACM5	Directed Mutation	N7	V					0.70	0.85		102
ACM5	Directed Mutation	N8, N10, N12	Q, S, N					0.40	1.13		102
ACM5	Directed Mutation	N5, N15	V, E					0.22	1.14		102
ACM5	Directed Mutation	N11	O					0.00	0.71		102
ACM5	Directed Mutation	N4	L					-0.04	1.35		102
ACM5	Directed Mutation	N5, N8, N12	L, O, A					-0.05	0.74		102
ACM5	Directed Mutation	N2, N10	L,S					-0.11	0.85		102
ACM5	Directed Mutation	N10, N15	I, E					-0.11	1.00		102
ACM5	Directed Mutation	N4, N6, N11, N14	W, A, M, G					-0.40	0.92		102
ACM5	Directed Mutation	N4, N17	W, L					-0.48	1.20		102
ACM5	Directed Mutation	N8, N13, N14	T, P, P					-0.76	0.79		102
ACM5	Directed Mutation	N1,N4,N5	O,O,A,V					-0.81	0.82		102
ACM5	Directed Mutation	N8,N15	D,R					-0.83	0.77		102
ACM5	Directed Mutation	N5	N,B					-0.89	0.72		102
ACM5	Directed Mutation	C10,C8,C7	G,K,S		agonist	0.04	IP3	0.40	1.10		103
ACM5	Directed Mutation	C21,C18,C13, C11-C7	R,P,PNRGS		agonist	0.09	IP3	0.40	1.20		103
ACM5	Directed Mutation	C15,C12	T,K				IP3	0.05	1.20		103
ACM5	Directed Mutation	C21,C9,C8	L,ET				IP3	-0.08	1.10		103
ACM5	Directed Mutation	C14,C3	I,K				IP3	-0.11	0.90		103
ACM5	Directed Mutation	C17,C16,C10	TQ,F				IP3	-0.15	1.20		103
ACM5	Directed Mutation	C17,C13-C11,C9	Q,GAO,R				IP3	-0.15	0.90		103
ACM5	Directed Mutation	C21,C19,C15, C12	R,I,N,I				IP3	-0.28	0.70		103
ACM5	Directed Mutation	C10,C9,C7	LT,T				IP3	-0.26	0.80		103
ACM5	Directed Mutation	C18,C17,C3	NG,H				IP3	-0.38	1.00		103
ACM5	Directed Mutation	C13,C12,C9,C6,C3,C2	GR,E,R,AM				IP3	-0.41	0.65		103
ACM5	Directed Mutation	C17,C9	G,E				IP3	-0.48	0.80		103
ACM5	Directed Mutation	C21,C20,C15, C13,C7	LP,R,E,S				IP3	-0.54	1.10		103
ACM5	Directed Mutation	C19,C12,C9	I,I,N				IP3	-0.62	1.00		103
ACM5	Directed Mutation	C17,C13,C9	G,S				IP3	-0.70	0.80		103
ACM5	Directed Mutation	C18,C17,C16, C14	R,QT,E				IP3	-0.95	0.80		103
ACM5	Directed Mutation	C20,C17,C16, C12,C5	P,NP,F,T				IP3	-0.95	1.00		103
ACM5	Directed Mutation	C19,C17,C10, C8,C6,C1	I,Q,R,V,H				IP3	-0.95	0.45		103
ACM5	Directed Mutation	C15,C12,C10	N,D,L				IP3	-1.11	0.80		103

ACM5	Directed Mutation	C20,C19,C12, C11,C9	PK,DO,				IP3	-1.20	1.20		103
ACM5	Directed Mutation	C18,C7,C3	I,T,P		agonist	0.11	IP3	-1.20	0.75		103
ACM5	Directed Mutation	C19,C18,C10, C5	IS,L,T				IP3	-1.20	0.90		103
ACM5	Directed Mutation	C21,C14,C11, C9,C8,C4,C2	Q,S,R,ND,T, K				IP3	-1.30	0.95		103
ACM5	Directed Mutation	C20,C2	H,P		agonist	0.16	IP3	-1.63	0.35		103
ACM5	Directed Mutation	C20,C19,C15-C13,C5,C3,C1	HI,MKL,Q,T, L,P				IP3	-1.65	0.5		103
ACM5	Directed Mutation	C10,C8-C6	L,DMT		agonist	-0.07	IP3	-1.81	0.55		103
ACM5	Directed Mutation	C21,C18,C12, C4,C2	Y,N,A,P,R				IP3	-1.88	0.5		103
ACM5	Directed Mutation	C13,C9,C4,C3	M,I,SP				IP3	-1.89	0.25		103
ACM5	Directed Mutation	C19,C14,C12, C7,C4	T,G,D,I,R				IP3	-1.94	0.7		103
ACM5	Directed Mutation	C19,C14,C13, C8	I,TE,D,N				IP3	-1.97	0.35		103
ACM5	Directed Mutation	C17,C15,C12, C10,C8,C4	R,M,F,G,D,T				IP3	-2.00	0.5		103
ADA1B	Deletion	N14-C12			agonist	0.60	IP1		0.80	$G\alpha_{16}$ Data	104
ADA1B	Deletion	N3-N5			agonist	0.01					104
ADA1B	Deletion	N6-C19			agonist	0.57					104
ADA1B	Deletion	N25-N32			agonist	0.02	IP1		0.20		104
ADA1B	Deletion	N25-C12			agonist	0.06					104
ADA1B	Deletion	N25-C5			agonist	0.37					104
ADA1B	Deletion	N3-N32			agonist	0.81					104
ADA1B	Directed Mutation	C6	L		agonist	0.09					104
ADA1B	Deletion,Directed Mutation	N25-N32,C6	L	C6	agonist	0.05					104
ADA1B	ICL3 replacement	N3-N5	**ADRB2**	N3-N5	agonist	0.99	IP3	0.08	0.55		105
ADA1B	ICL3 replacement	N18-N26	**ADRB2**	N10-N18	agonist	0.26	IP3	-0.33	0.55		105
ADA1B	ICL3 replacement	N28-N35	**ADRB2**	N17-N24	agonist	0.10	IP3		0.09		105
ADA1B	ICL3 replacement	C35-C30	**ADRB2**	N27-C24	agonist	0.16	IP3	0.37	0.67		105
ADA1B	ICL3 replacement	C9-C3	**ADRB2**	C9-C3	agonist	2.29	IP3	2.54	1.06		105
ADA1B	Directed Mutation	C9	**ADRB2**	C9	agonist	0.21	IP3	0.10	1.00		105
ADA1B	Directed Mutation	C7	**ADRB2**	C7	agonist	1.25	IP3	1.70	0.94		105
ADA1B	Directed Mutation	C4	**ADRB2**	C3	agonist	1.89	IP3	2.24	0.86		105
ADA1B	Directed Mutation	C4	T		agonist	1.11	IP3	1.21	0.95		106
ADA2A	ICL3 replacement	N(-19)-C(-6)	**ADRB2**	N(-2)-C(-19)	agonist	-1.66					1
ADA2A	ICL3 replacement	N1-N18	**ADRB2**	N1-N18	agonist	-0.07	cAMP Inhibition	-0.43	1.26		107
ADA2A	ICL3 replacement	N1-N18	**5HT1A**	N1-N18	agonist	0.14	cAMP Inhibition	-0.08	1.08		107
ADA2A	ICL3 replacement	N1-N18, C22-C6	**ADRB2**	N1-N18, C22-C6	agonist	-0.13	cAMP Inhibition	-1.81	0.87		107
ADA2A	ICL3 replacement	N1-N18, C22-C6	**5HT1A**	N1-N18, C22-C6	agonist	-0.16	cAMP Inhibition	-0.18	1.26		107
ADA2A	ICL3 replacement	C22-C6	**ADRB2**	C22-C6	agonist	0.56	cAMP Inhibition	0.18	0.79		107
ADA2A	ICL3 replacement	C22-C6	**5HT1A**	C22-C6	agonist	-0.04	cAMP Inhibition	-0.18	1.06		107
ADA2A (pig)	Directed Mutation	C16,C14,C12	A,A,A		agonist	0.50	cAMP inhibition	-0.12	1.02		108
ADA2A (pig)	Directed Mutation	C7,C6	A,A		agonist	0.50	cAMP inhibition	-1.30	0.96		108

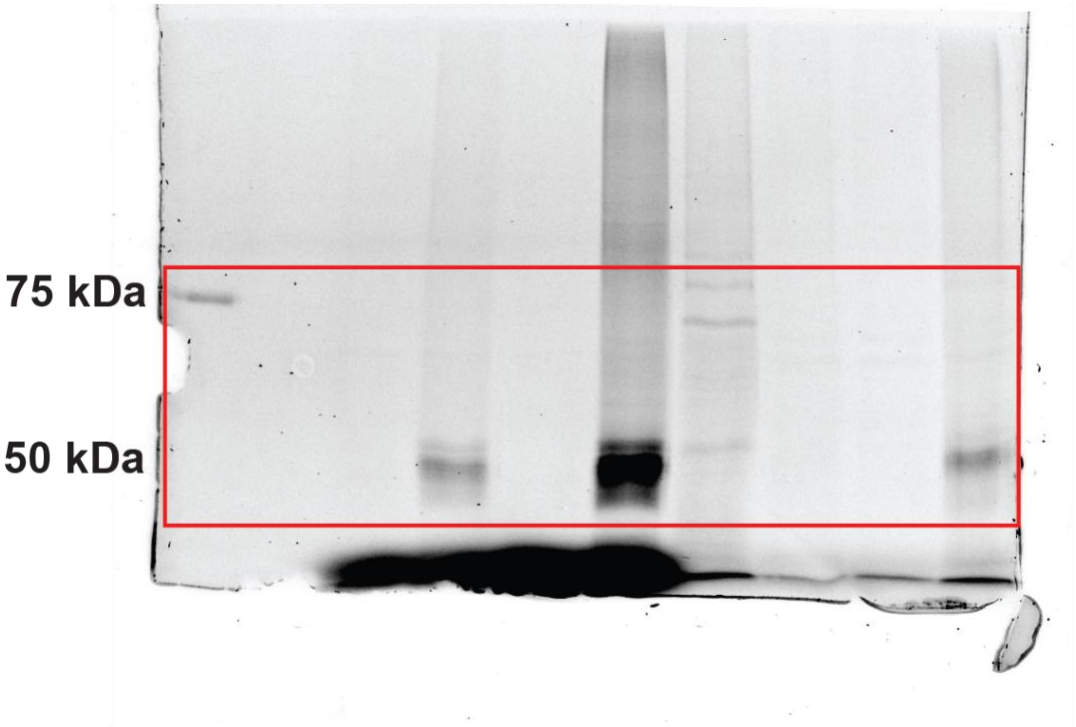
ADA2A (pig)	Directed Mutation	C9,C7,C6	A,A,A		agonist	0.50	cAMP inhibition	-1.69	0.84		108
ADA2A (pig)	Directed Mutation	C9,C7,C6	A,A,A		antagonist	0.08	cAMP inhibition	-2.27	0.75		108
ADRB2	ICL3 replacement	N4-C4	**ADA1B**	N4-C4	agonist	0.63	cAMP	-0.48	0.28		109
ADRB2	ICL3 replacement	N4-N20,C9-C4	**ADA1B**	N4-N20,C9-C4	agonist	1.53	cAMP	-0.26	0.21		105
ADRB2	ICL3 replacement	N4-N13,C9-C4	**ADA1B**	N4-N13,C9-C4	agonist	1.51	cAMP	-0.22	0.26		105
ADRB2	ICL3 replacement	N9-N24	**ADA1B**	N9-N24	agonist	0.63	cAMP	-0.91	0.83		105
ADRB2	ICL3 replacement	N(-1)-N7	**ACM1**	N(-1)-N10	agonist	0.70	cAMP		0.01		110
ADRB2	Directed Mutation	C17	G		agonist	0.66	cAMP	0.65	0.96		2
ADRB2	Directed Mutation	C18-C21	KRFI		agonist	-0.19	cAMP	-0.54	0.78		2
ADRB2	Deletion	C9-C3			agonist	0.49	cAMP	-0.46	0.54		2
ADRB2	Deletion	C13-C3			agonist	0.06	cAMP	-0.23	0.83		2
ADRB2	Deletion, Directed Mutation	C9-C(-4)	VLAVVI								2
ADRB2	Deletion	N1-N7	E,F								2
ADRB2	Deletion	N2-N8									2
ADRB2	Directed Mutation	N(-5)-N5	ILVYVRIYQI	N(-5)-N5	agonist	0.92	cAMP	0.50	0.77		2
ADRB2	Deletion	C9-C3			agonist	-0.05	cAMP		0.46	Derived from Table 1 (Iso/FSK) and Table 2 (cyclase mix)	111
ADRB2	Insertion	N10-C13	**T_4L**		agonist	0.51					112
ADRB2	Deletion	N16-C13			agonist	-0.19					113
ADRB2	Insertion	N13-C9	**T_4L**		agonist	0.60					24
ADRB2	Directed Mutation	C10,C9,C7,C4	S,R,K,A		agonist	1.34	cAMP	0.89	1.00	+GppNhp condition	114
ADRB2 (hamster)	Deletion	N9-N16			agonist	-0.54	cAMP	0.60	1.11		115
ADRB2 (hamster)	Deletion	N19-C4			agonist	0.58	cAMP				115
ADRB2 (hamster)	Deletion	N9-N16			agonist	-0.41	cAMP	-0.18	0.67		115
ADRB2 (hamster)	Deletion	N19-C4			agonist	-0.40	cAMP	-0.40	0.67		115
ADRB2 (hamster)	Deletion	C4-C(-1)			agonist	-0.40	cAMP	-0.30	0.89		115
ADRB2 (hamster)	Directed Mutation	N7, N8, N12, N15	S,S,S,S		agonist	-0.60	cAMP	-0.37	0.77		116
ADRB2 (hamster)	Directed Mutation	N7, N12, N15	S,S,S		agonist	-0.85	cAMP	-0.52	0.87		116
ADRB2 (hamster)	Directed Mutation	N2, N3, N5, N6, N13	L,L,L,L,L		agonist	0.40	cAMP	-0.48	0.30		116
ADRB2 (hamster)	Directed Mutation	N1, N2, N3, N4, N5, N6, N7, N8, N9	K,A,L,A,A,L, A,K,K		agonist	-0.78	cAMP	-0.43	0.20		116
ADRB2 (hamster)	Deletion	N(-1)-N7			agonist	1.46	cAMP		0.31	+GppNhp condition	7
ADRB2 (hamster)	Deletion	N7-N14			agonist	-0.18	cAMP	0.53	0.97	+GppNhp condition	7
ADRB2 (hamster)	Deletion	N16-N29			agonist	1.82	cAMP	0.28	1.97	+GppNhp condition	7
ADRB2 (hamster)	Deletion	N21-C6			agonist	1.82	cAMP		0.31	+GppNhp condition	7
ADRB2 (hamster)	Deletion	N28-C19			agonist	1.00	cAMP	-0.25	0.94	+GppNhp condition	7
ADRB2 (hamster)	Deletion	C20-C8			agonist	-0.30	cAMP		0.41	+GppNhp condition	7
ADRB2 (hamster)	Deletion	N8-C12			agonist	-0.40	cAMP	-1.60	0.50	+GppNhp condition	117
AGTR1	Directed Mutation	N4,N7,N8,N10	Q,Q,Q,F		agonist	-0.37	IP3		1.13		118

AGTR1	Directed Mutation	C13,C11,C9,C7,C6	Q,Q,Q,G,G		agonist	0.00	IP3		0.77		[118]
AGTR1	ICL3 replacement	N2-C7	**AGTR2**	N2-C7	agonist	-0.06	gene expression		0.05		[119]
AGTR1	ICL3 replacement	N10-C7	**AGTR2**	N10-C7	agonist	0.12	gene expression		0.47		[119]
AGTR1	ICL3 replacement	N3-N9	**AGTR2**	N3-N9	agonist	0.16	gene expression		0.30		[119]
AGTR1	ICL3 replacement	N3-N10	**AGTR2**	N3-N10	agonist	-0.07	gene expression		0.06		[119]
AGTR1	ICL3 replacement	N3-C10	**AGTR2**	N3-C10	agonist	0.11	gene expression		0.24		[119]
AGTR1	ICL3 replacement	C13-C7	**AGTR2**	C13-C7	agonist	0.09	gene expression		0.54		[119]
AGTR1	ICL3 replacement	N10-C10	**AGTR2**	N10-C10	agonist	0.02	gene expression		0.92		[119]
C5AR1	Directed Mutation	N7					gene expression		0.55		[120]
C5AR1	Directed Mutation	N12					gene expression		0.40		[120]
C5AR1	Directed Mutation	C9					gene expression		0.25		[120]
C5AR1	Directed Mutation	C8					gene expression		0.20		[120]
C5AR1	Directed Mutation	C6					gene expression		0.10		[120]
CCR2	ICL3 replacement	N(-1)-C1	**CXCR1**	N(-1)-C1			IP3		0.08		[121]
CXCR4	Insertion	N9-N10	**T_4L**		antagonist	-0.31					[122]
CXCR4	Insertion	N9-N10	GS-**T_4L**-GS		antagonist	-0.33					[122]
DRD2	Directed Mutation	N23	G		agonist	0.04	cAMP inhibition	-0.12	0.94		[123]
DRD2	Directed Mutation	N24	T		agonist	-0.06	cAMP inhibition	-0.12	1.09		[123]
DRD2	Deletion	N32-N60			antagonist	-0.13	cAMP inhibition	0.25	1.00		[124]
DRD2	Directed Mutation	N41	V		antagonist	0.11	cAMP inhibition	-0.13	1.02		[124]
DRD2	Directed Mutation	N48,N57-N59	V,V,V,V		antagonist	0.13	cAMP inhibition	-0.45	1.03		[124]
DRD2	Directed Mutation	N48,N57-N59,N64,N65	V,V,V,V,V,V		antagonist	0.19	cAMP inhibition	-0.40	0.81		[124]
DRD2	Directed Mutation	N54	G		antagonist	-0.05	cAMP inhibition	-0.21	1.02		[124]
DRD2	Directed Mutation	N49,N52	A,A		antagonist	0.03	cAMP inhibition	0.43	0.96		[124]
DRD2	Directed Mutation	N39	V		antagonist	-0.10	cAMP inhibition	0.72	0.93		[124]
DRD2	Directed Mutation	N61	V		antagonist	0.04	cAMP inhibition	-0.02	0.96		[124]
DRD2	Deletion	N32-N60			agonist	0.05	cAMP inhibition		1.17		[125]
DRD3	Insertion	N13-C14	**T_4L**		agonist	-0.47					[126]
FFAR1	Insertion	N9-N10	**T_4L**		agonist	-0.03				additional mutations	[127]
FFAR1	Insertion	N9-N10	GS-**T_4L**-GS		agonist	0.10				additional mutations	[127]
GASR (rat)	Directed Mutation	C8	M		agonist	0.09	IP3		0.71		[128]
GASR (rat)	Directed Mutation	C7	T		agonist	-0.04	IP3		0.24		[128]
GASR (rat)	Directed Mutation	C7	L		agonist	-0.10	IP3		0.07		[128]
GLP1R	Deletion	N12-N14			agonist	0.08	cAMP		0.80	Also performed point mutations in this region	[129]
GLP1R	Deletion	N9-N11			agonist	0.07	cAMP		0.10		[129]
GLP1R	Deletion	N6-N8			agonist	0.33	cAMP		0.05		[129]
GLP1R	Deletion	N4-N5			agonist	0.22	cAMP		0.20		[129]
GLP1R	Deletion	N2-N3			agonist	0.13	cAMP		0.05		[129]
HRH1	Insertion	N11-C14	**T_4L**		agonist	0.07					[130]

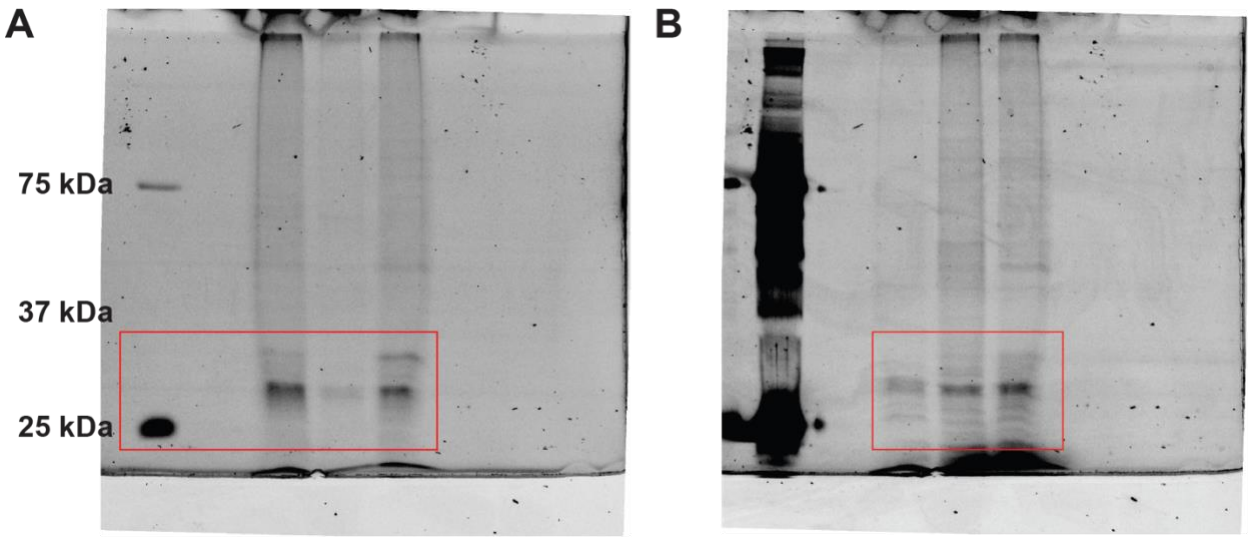
NMBR	ICL3 replacement	N3-C4	**ACM3**	N1-C2	agonist	0.08	IP3		0.08		131
NTS1	Insertion	N11-C12	**T_4L**		agonist	-0.06				additional mutations	132
NTS1	Deletion	N19-C16	**T_4L**		agonist	0.09	GTPγS turnover		0.40	additional mutations	133
NTS1	Insertion	N11-C6	**T_4L**		agonist	-0.06				additional mutations	134
OPN2	ICL3 replacement	N8-C12	**ADRB2**	N8-C12			G_t activation		0.08		135
OPN2	ICL3 replacement	N9-C12	**ADRB2**	N8-C12			G_t activation		0.1		
OPRK	Insertion	C16-C14	**T_4L**		agonist	0.14				I135L mutation	136
OPRM	Insertion	N11-C15	**T_4L**		antagonist	0.04					137
OPSD (bovine)	Deletion	N12-C6					GtPase Activity		0.00		138
OPSD_bovine	ICL3 replacement	N6-C3	**PTGER3 (mouse)**	N7-C3			G_t activation		0.1		139
OXTR	ICL3 replacement	N4-C3	**V2R**	N4-C3	agonist	-1.03					140
OXTR	Directed Mutation	C10	A		agonist	-0.18	IP3	-0.08	2622.00		140
OXTR	Directed Mutation	C8	V		agonist	-0.69					140
OXTR	Directed Mutation	C10,C8	A,V		agonist	-0.80					140
P2Y12	Insertion	N13	**BRIL**		agonist	-0.09					141
PACR	Insertion	N7	TNLRLRVPKKTREDPLPVPSDQHSPPFL		agonist	-0.30	IP3	-0.10			142
PACR	Insertion	N7	SCVQKCYCKPQRAQQHSCKMSELSTITL		agonist	0.00	IP3	-0.10			142
PTH1R	Directed Mutation	N4-N7	AAAA		agonist	2.86	cAMP	-0.66	0.63		143
PTH1R	Directed Mutation	N8-N11	AAAA		agonist	3.23	cAMP	-0.64	0.40		143
PTH1R	Directed Mutation	N12-C17	AAAA		agonist	3.01	cAMP	-0.08	0.85		143
PTH1R	Directed Mutation	C16-C13	AAAA		agonist	3.02	cAMP	0.33	1.02		143
STE3	Deletion	N12-N14					gene expression		0.56		144
STE3	Deletion	N15-C20					gene expression		2.08		144
STE3	Deletion	N19-C17					gene expression		2.22		144
STE3	Deletion	C20-C15					gene expression		1.94		144
STE3	Deletion	C16-C11					gene expression		1.94		144
STE3	Deletion	C12-C7					gene expression		0.56		144
TSHR	Directed Mutation	C10-C6,C3,C2	NQNAG,Q,G		agonist	-0.93	cAMP		0.84		145
TSHR	Directed Mutation	N1,N3,N5,N7, C14	G,A,A,G,A		agonist	0.25	cAMP	0.39	0.21		145
TSHR (rat)	ICL3 replacement	C4	K		agonist	0.92	cAMP	-0.04	0.73	E mutation at same position had similar effects	146
TSHR	ICL3 replacement	N5-N7	**ADA1A**	N5-N7	agonist	0.04	cAMP	-0.22	0.69		147
TSHR	ICL3 replacement	N3-N7	**ADRB2**	N3-N7	agonist	0.09	cAMP	-0.48	0.74		147
TSHR	ICL3 replacement	N8-N11	**ADA1A**	N8-N11	agonist	0.07	cAMP	0.04	0.97		147
TSHR	ICL3 replacement	C10-C7	**ADA1A**	C11-C8	agonist	0.85	cAMP	-0.67	0.86		147
TSHR	ICL3 replacement	C5-C2	**ADA1A**	C6-C3	agonist	-0.02	cAMP	-0.63	0.60		147
TSHR	ICL3 replacement	C6-C2	**ADRB2**	C7-C3	agonist	0.54	cAMP	-0.80	0.57		147

TSHR	ICL3 replacement	N8-N11	**ADRB2**	N8-N11	agonist	0.32	cAMP	-0.03	0.91		147
TSHR	ICL3 replacement	C10-C7	**ADRB2**	C11-C8	agonist	0.85	cAMP	-0.55	1.03		147
V1AR (rat)	ICL3 replacement	N(-1)-C(-1)	**V2R**	N(-1)-C(-1)	agonist	0.30	cAMP	-0.29	0.76		148
V1AR (rat)	ICL3 replacement	N(-1)-C22	**V2R**	N(-1)-C22	agonist	-0.16	cAMP	-0.33	0.71		148
V1AR (rat)	ICL3 replacement	N13-C(-1)	**V2R**	N13-C(-1)	agonist	0.32	cAMP		0.11		148
V1AR (rat)	ICL3 replacement	N(-1)-N10	**V2R**	N(-1)-N10	agonist	-0.27	cAMP	-0.86	0.29		148
V1AR (rat)	ICL3 replacement	N(-1),N1	**V2R**	N(-1),N1			cAMP		0.10		148
V1AR (rat)	ICL3 replacement	N4-N6	**V2R**	N4-N6			cAMP		0.05		148
V1AR (rat)	ICL3 replacement	N8-N10	**V2R**	N8-N10			cAMP		0.04		148
V1AR (rat)	ICL3 replacement	N(-1),N1,N13-C(-1)	**V2R**	N(-1),N1,N13-C(-1)			cAMP		0.20		148
V1AR (rat)	ICL3 replacement	N4-N6,N3-C(-1)	**V2R**	N4-N6,N3-C(-1)	agonist	-0.01	cAMP	-0.86	0.44		148
V1AR (rat)	ICL3 replacement	N8-N10,N13-C(-1)	**V2R**	N8-N10,N13-C(-1)			cAMP		0.09		148
V2R	ICL3 replacement	N4-C3	**OXTR**	N4-C3	agonist	-0.06					140
V2R	ICL3 replacement	N4-N16	**OXTR**	N4-N16	agonist	-0.75	cAMP		0.44		140
V2R	ICL3 replacement	N19-C3	**OXTR**	N19-C3	agonist	-0.21	cAMP	-0.30	0.85		140

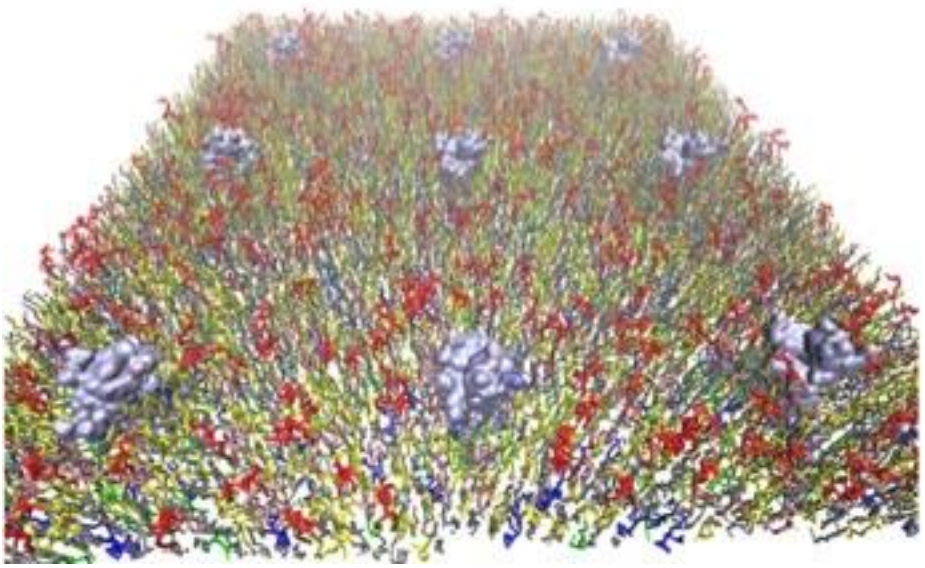
Supplementary Table 1. Effect of ICL3 mutagenesis on receptor pharmacology. Column 1, Receptor gene name (Uniprot ID). Column 2, Type of mutation: Insertion refers to insertion of a protein domain, deletion refers to truncation of amino acids, ICL3 replacement refers to switching ICL3 domain from one receptor to another, directed mutation refers to mutagenesis of individual amino acid(s). Column 3, Location of mutation: Nx refers to sequence position relative to TM5.56, read N-C. Cx refers to sequence position relative to TM6.37, read C-N. Column 4, new sequence. Entries in bold reflect new domains or new ICL3 sequences. T_4L refers to T_4-lysozyme, BRIL refers to apocytochrome b_{562}RIL. Column 6, Positions of new ICL3 sequences (for ICL3 replacement mutations), using the same numbering scheme as column 3. Column 4, ligand class used for binding assays (agonist vs antagonist). Column 5, comparison of ligand binding for the wild-type receptor versus the mutant. Column 6, Assay: refers to the measurement technique for assessing signaling potency (EC_{50}) and/or efficacy (E_{max}). Measurements curated are for canonical signaling for the wild-type receptor. Column 7, comparison of signaling potency for the wild-type receptor versus the mutant. Column 8, comparison of signaling efficacy for the wild-type receptor versus the mutant. Column 9, notes on which values are curated and/or additional mutations noted for the receptor which may affect changes in receptor function. Column 10, reference from which data are derived.



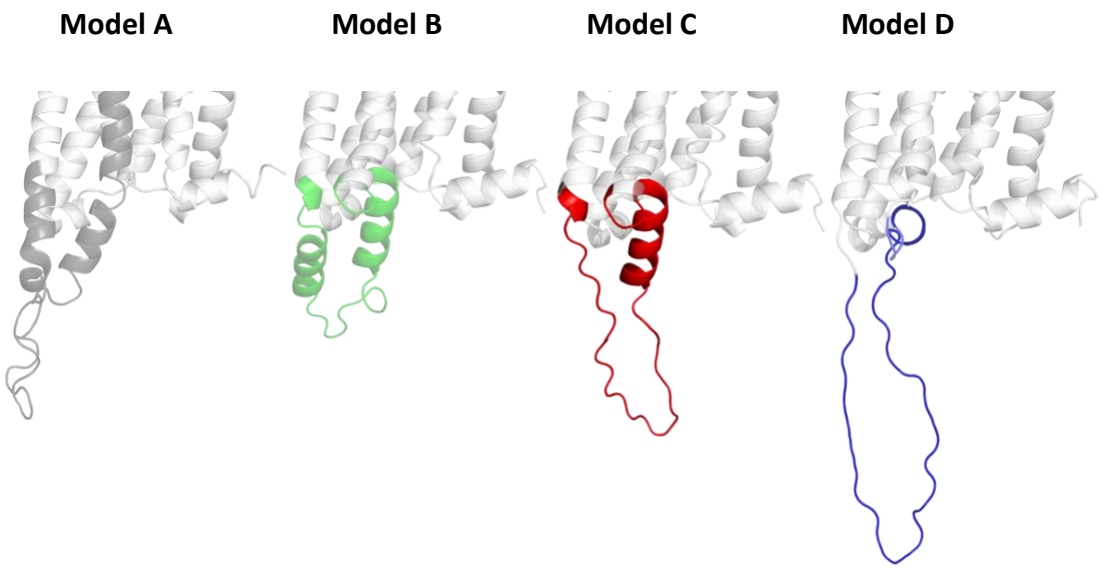
Supplementary Figure 1. Uncropped image from Extended Data Figure 3e. Boxes refer to the cropped areas in the figure, band in leftmost lane (75 kDa) is from the autofluorescence of ladder.



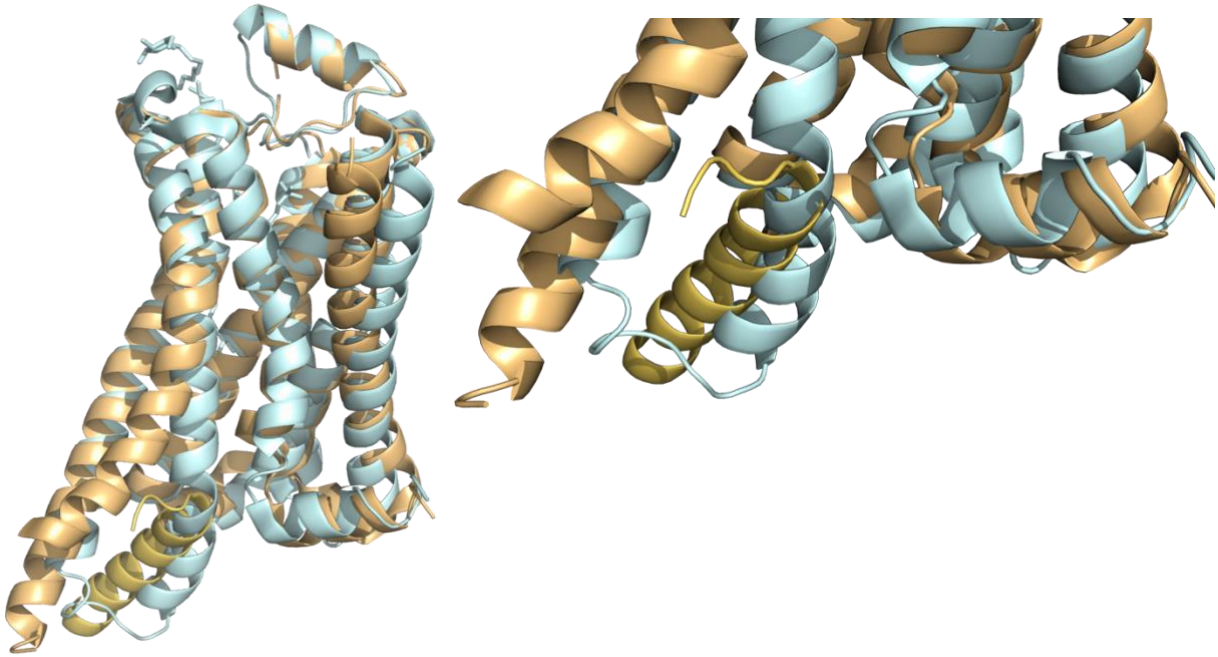
Supplementary Figure 2. Uncropped image from Extended Data Figure 4n. (**A**) is the 488 gel scan, and (**B**) is the 546 gel scan. The 10% polyacrylamide gel matrix prevents complete migration of labeled bands, evident in the signal at the interface of stacking and resolving gels (top of the lanes). Lower % acrylamide facilitates entry of fluorescent protein (note the lack of bands at the stacking-resolving interface in Supplementary Figure 1). However, it does not resolve the lower molecular weight bands. Boxes refer to the cropped areas in the figure. Bands at 75 kDa and 25 kDa in the leftmost lane is from the auto-fluorescent ladder (labeled).



Supplementary Figure 3. Starting model of the cell membrane consisting of multiple lipids used in the coarse grain simulations to equilibrate the multi-lipid bilayer[22].



Supplementary Figure 4. Structural models of ICL3 in β_2AR used as starting structures for MD simulations in cell membrane conditions.



Supplementary Figure 5. β_1AR inactive state structure displays cytoplasmic TM6 conformation similar to the Gα_s C-terminus (G_s peptide). Left: Overlay of the inactive state structure of β_1AR (pale cyan) (PDB ID: 2ycx)[23] with the fully active state structure of β_2AR (orange) bound to the G_s protein (PDB ID: 3sn6)[17]. The last 24 amino acids of the Gs protein are shown (gold). Right: Close up view of the two structures showing that the intracellular part of helix 6 and ICL3 blocks the G protein binding site in the inactive state.

Starting Structure used for MD simulations	Number of simulations	Simulation time for each velocity (μs)	Total simulation time (μs)
Model A	5	0.4	2
Model B	5	0.4	2
Model C	5	0.4	2
Model D	25	0.4	10
*Starting structure: snapshot at end of 50 ns (Model D).	5	0.4	2
*Starting structure: snapshot at end of 100 ns (Model D).	5	0.4	2
*Starting structure: snapshot at end of 150 ns (Model D).	5	0.4	2

Supplementary Table 2. Simulation parameters (see Methods for details). A swarm of MD simulations, starting from each structural template of ICL3, was used to create the conformational landscape of ICL3 spanning. Bottom 3 rows reflect simulations starting from timepoints within the “Model D” simulation trajectories. These were used to better sample transitions between “intermediate” and “open” states of ICL3.

Receptor	Sequence	Ligand
β_2AR WT	FVYSRVFQEAKRQLQKIDKSEGRFHVQNLSQVEQDGRTGHGLRRSSKFCLKEHKALKTL	Isoproterenol (100 μM), Metoprolol (100 μM) for FSK Inhibition
β_2AR ΔICL3	FVYSRVFQEAKRQLQKIDK----------KFCLKEHKALKTL	
β_1AR WT	FVYLRVFREAQKQVKKIDSCERRFLGGPARPPSPSPSPVPAPAPPPGPPRPAAAAATAPLANGR AGKRRPSRLVALREQKALKTL	Isoproterenol (100 μM), Metoprolol (100 μM) for FSK Inhibition
β_1AR ΔICL3	FVYLRVFREAQKQVKKID---------- AGKRRPSRLVALREQKALKTL	
D_1R WT	VTYTRIYRIAQKQIRRIAALERAAVHAKNCQTTTGNGKPVECSQPESSFKMSFKRETKVLKTL	Dopamine (100 μM)
D_1R ΔICL3	VTYTRIYRIAQKQIRRIAA----------PESSFKMSFKRETKVLKTL	
M_1R WT	TLYWRIYRETENRARELAALQGSETPGKGGGSSSSSERSQPGAEGSPETPPGRCCRCCRAPRLLQAYSWKEE EEEDEGSMESLTSSEGEEPGSEVVIKMPMVDPEAQAPTKQPPRSSPNTVKRPTKKGRDRAGKGQKPRGKEQL AKRKTFSLVKEKKAARTL	Carbachol (100 μM)
M_1R ΔICL3	TLYWRIYRETENRARELAA---------- ---------- AKRKTFSLVKEKKAARTL	
V_{1A}R WT	TCYGFICYNIWCNVRGKTASRQSKGAEQAGVAFQKGFLLAPCVSSVKSISRAKIRTVKMT	Arginine vasopressin (100 nM)
V_{1A}R ΔICL3	TCYGFICYNIWCNVRGKTA----------VSSVKSISRAKIRTVKMT	
A_{1A}R WT	LIYLEVFYLIRKQLNKKVSASSGDPQKYYGKELKIAKSL	N_6-methyladenosine (10 μM)
A_{1A}R ΔICL3	LIYLEVFYLIRKQLNKKV-------QKYYGKELKIAKSL	
CB_1R WT	YAYMYILWKAHSHAVRMIQRGTQKSIIIHTSEDGKVQVTRPDQARMDIRLAKTL	2-Arachidonoylglycerol (10 μM)
CB_1R ΔICL3	YAYMYILWKAHSHAVRMIQ----------QVTRPDQARMDIRLAKTL	

Supplementary Table 3. Receptor ICL3 truncations. Column 2 is the ICL3 sequence (TM5.56-TM6.37). Column 3 is the saturating agonist concentration used for signaling assays.

Insertion	Key		Inserted Sequence	Gs signal	N	Gq signal	N
Wild-Type				0.56±0.13	15	0.18±0.08	12
CB_2R	G_i	1	GHQDRQ	0.55±0.06	3	0.16±0.02	3
CB_1R	G_i	2	RGTQKSIIIHTSEDGKV	0.53±0.12	3	0.10±0.07	3
D_3R	G_i	3	TRQNSQCNSVRPGFPQQTLSPDPAHLELKRYYSICQDTALGGP GFQERGGELKREEKTRNSLSPTIAPKLSLEVRKLSNGRLSTSL KLG	0.72±0.11	5	0.08±0.17	3
5HT1D	G_i	4	LYGKRFTTAHLITGSAGSSLCSLNSSLHEGHSHSAGSPLFFNH VKIKLADSA	0.24±0.05	3	-0.15±0.12	3
M_2R	G_i	5	EPVANQDPVSPSLVQGRIVKPNNNNMPSSDDGLEHNKIQNGKA PRDPVTENCVQGEEKESSNDSTSVSAVASNMRDDEITQDENTV STSLGHSKDENSKQTCIRIGTKTPKSDSCTPTNTTVEVVGSSG QNGDEKQNIVARKIVKMTKQ	0.35±0.09	3	0.10±0.10	5
β_1AR	G_s	1	CERRFLGGPARPPSPSPSPVPAPAPPPGPPRPAAAAATAPLAN GRAGK	0.47±0.07	4	0.06±0.03	3
D_1R	G_s	2	LERAAVHAKNCQTTTGNGKPVECSQP	0.58±0.13	5	0.13±0.14	4
β_2AR	G_s	3	SEGRFHVQNLSQVEQDGRTGHG	0.56±0.17	4	0.1±0.09	4
H_1R	G_q	1	INRSLPSFSEIKLRPENPKGDAKKPGKESPWEVLKRKPKDAGG GSVLKSPSQTPKEMKSPVVFSQEDDREVDKLYCFPLDIVHMQA AAEGSSRDYVAVNRSHGQLKTDEQGLNTHGASEISEDQMLGDS QSFSRTDSDTTTETAPGKGKLRSGSNTGLDYIKFTWKRLRSHS R	0.66±0.13	3	-0.09±0.08	3
5HT2A	G_q	2	DLGTRAKLASFSFLPQSSLSSEKLFQRSIHREPGSYT	0.37±0.07	3	0.02±0.05	3
$\alpha_{1A}AR$	G_q	3	GLKTDKSDSEQVTLRIHRKNAPAGGSGMASAKTKTH	0.59±0.19	4	0.14±0.06	4
GPR27	G_q	4	MRPARLVPAVSHDWTFHGPGATGQAAANWTAGFGRGPTPPALV GIRPAGPGRGAR	0.79±0.1	3	0.14±0.06	3
M_1R	G_q	5	LQGSETPGKGGGSSSSSERSQPGAEGSPETPPGRCCRCCRAPR LLQAYSWKEEEEEDEGSMESLTSSEGEEPGSEVVIKMPMVDPE AQAPTKQPPRSSPNTVKRPTKKGRDRAGKGQKPRGKEQL	0.44±0.13	5	0.26±0.07	3
CCK_2R	G_q	6	SDSDSQSRVRNQGGLPGAVHQNGRCRPETGAVGEDSDGCYVQL PRSRPALELTALTAPGPGSG	0.42±0.06	4	0.12±0.14	4
$V_{1A}R$	G_q	7	SRQSKGAEQAGVAFQKGFLLAPC	0.33±0.04	3	0.01±0.13	3
5HT2C	G_q	8	GHTEEPPGLSLDFLKCCKRNTAEEENSANPNQDQNARRRKKKE RR	0.48±0.08	3	0.00±0.08	3
NTS_1R	G_q	9	QGQVCTVGGEHSTFS	0.48±0.14	6	0.14±0.18	6
NTS_2R	G_q	10	TSTPGSSTPSRLELLSEEGLLSFIVWKKTFIQGGQVSLV	0.36±0	4	-0.10±0.12	3

Supplementary Table 4. Sequence information and data from PTH1R-ICL3 insertion screen (Figure 5). Column 1, names of host receptors from which inserted ICL3s are derived. Wild-Type indicates Wild-Type PTH_1R. Columns 2-3, key for Figure 5F and G. Column 2 indicates primary G protein subtype of host receptor. Column 4, inserted ICL3 sequence. Column 5, specific G_s-peptide luminescence signal (mean ± standard deviation). Column 6, number of biological replicates for G_s-peptide assay. Column 7, specific G_q-peptide luminescence signal (mean ± standard deviation). Column 8, number of biological replicates.

Receptor	Positions	Insertion	Positions	Assay	pEC50 (Wild-Type)	pEC50 (Mutant)	Emax (Wild-Type)	Emax (Mutant)	Notes	Reference
ACM1	N25-C11	**ADRB1 (turkey)**	N22-C11	cAMP			1	49	derived from table 2 - PTX data	81
ACM2	N(-7)-C1	**ACM1**	N(-7)-C2	current response			210	2910		82
ACM2	N1-C2	**ACM3**	N1-C3	IP3	7.8	9.3	41	233		3
ACM2	N1-N16	**ACM3**	N1-N17	IP3	7.8	8.3	41	137		3
ACM2	N1-C2	**ACM3**	N1-C3	IP3	8.6	7.8	61	73		90
ACM2	N1-N16	**ACM3**	N1-N17	IP3	8.6	7.7	61	26		90
ACM2	N1-N25	**ADRB1 (Turkey)**	N1-N26	cAMP			0	400		94
ACM2	N1-N21	**ACM3**	N1-N21	IP3		5.54	11	40		95
ACM3	N1-C3	**ACM2**	N1-C2	cAMP Inhibition	-	9.9	1	18		90
ACM3	N1-N17	**ACM2**	N1-N16	cAMP Inhibition	-	10.3	1	11		90
ACM3	N11-C4	**GPR183**	N12-C9	G_{12} dissociation			0	0.34	Mutations-Y149C and A239G	37
ACM3	N11-C5	**NMBR**	N12-C9	G_{12} dissociation			0	0.08	Mutations-Y149C and A239G	37
ACM3	N11-C6	**S1PR2**	N12-C9	G_{12} dissociation			0	0.07	Mutations-Y149C and A239G	37
ACM3	N11-C7	**GPR132**	N12-C9	G_{12} dissociation			0	0.29	Mutations-Y149C and A239G	37
ACM3	N11-C8	**LTB4R2**	N12-C9	G_{12} dissociation			0	0.17	Mutations-Y149C and A239G	37
ACM3	C3-C(-1)	**ACM2**	C3-C(-1)	cAMP Inhibition			1	30		96
ACM3	N1-N16, C3-C(-1)	**ACM2**	C3-C(-1)	cAMP Inhibition			1	35		96
ADA2A	N(-19)-C(-6)	**ADRB2**	N(-2)-C(-19)	cAMP	-	6.3	0	35		1
ADRB2	N4-C4	**ADA1B (Hamster)**	N4-C4	IP3	-	9.4	0	162		149
ADRB2	N4-N20,C9-C4	**ADA1B (Hamster)**	N4-N20,C9-C4	IP3	-	11.0	0	155		149
ADRB2	N4-N20	**ADA1B (Hamster)**	N4-N20	IP3	-	10.5	0	135		149
ADRB2	N4-N13,C9-C4	**ADA1B (Hamster)**	N4-N13,C9-C4	IP3			0	10		149
ADRB2	N19-N24	**ADA1B (Hamster)**	N19-N24	IP3			0	10		149
ADRB2	N14-N24	**ADA1B (Hamster)**	N14-N24	IP3			0	9		149
ADRB2	N9-N24	**ADA1B (Hamster)**	N12-N24	IP3			0	12		149
ADRB2	N9-N24	**ADA1B (Hamster)**	N9-N24	IP3	-	9.4	0	110		149
ADRB2	N9-N18	**ADA1B (Hamster)**	N9-N18	IP3			0	18		149
AGTR2	N2-C7	**AGTR1**	N2-C7	gene expression			20	60		119
CXCR1	N(-1)-C1	**CCL2**	N(-1)-C1	IP3			100	300		121
OPN2	N1-C4	**ADRB2**	N1-C4	cAMP			0	0.61		135
OPSD (bovine)	N6-C3	**mAChR1 (pig)**	N7-C3	Gq activation			-	5		139
OPSD (bovine)	N6-C3	**mAChR2 (pig)**	N7-C3	Gi activation			-	140		139
OPSD (bovine)	N6-C3	**ADA1B (hamster)**	N7-C3	Gq activation			-	5		139
OPSD (bovine)	N6-C3	**ADA2B (human)**	N7-C3	Gi activation			-	40		139
OPSD (bovine)	N6-C3	**ADRB2 (human)**	N7-C3	Gs activation			-	40		139
OPSD (bovine)	N6-C3	**PTGER2 (mouse)**	N7-C3	Gs activation			-	1		139
OPSD (bovine)	N6-C3	**PTGER3 (mouse)**	N7-C3	Gi activation			-	50		139

OPSD (bovine)	N6-C3	**EDNRA (human)**	N7-C3	G protein activation			-	140		[139]
OPSD (bovine)	N6-C3	**EDNRB (human)**	N7-C3	G protein activation			-	125		[139]
OPSD (bovine)	N6-C3	**ADRB2 (human)**	N7-C3	Gs activation			-	40		[139]
PACR (rat)	N24-C23	**PAFR (rat)**	N(-2)-C1	IP3			1000	5000		[150]
V1AR (rat)	N13-C(-1)	**V2R**	N13-C(-1)	IP3			-	70		[148]
V1AR (rat)	C16-C(-1)	**V2R**	N(-1)-N10	IP3			-	72		[148]
V1AR (rat)	N8-N10	**V2R**	N8-N10	IP3			-	75		[148]
V1AR (rat)	N(-1),N1,N13-C(-1)	**V2R**	N(-1),N1,N13-C(-1)	IP3			-	100		[148]
V1AR (rat)	N8-N10,N13-C(-1)	**V2R**	N8-N10,N13-C(-1)	IP3			-	100		[148]
V2R	N4-C3	**OXTR**	N4-C3	IP3	8.49	9.21	1019	3714		[140]
V2R	N19-C3	**OXTR**	N19-C3	IP3	8.49	8.19	1019	2668		[140]

Supplementary Table 5. **Gain-of-function for new signaling pathways by ICL3 mutagenesis**. Column 1, Receptor gene name (Uniprot ID). Column 2, Location of mutation: Nx refers to sequence position relative to TM5.56, read N-C. Cx refers to sequence position relative to TM6.37, read C-N. Column 3, new sequence. Column 4, Positions of new ICL3 sequences (for ICL3 replacement mutations), using the same numbering scheme as column 3. Column 5, Assay: refers to the measurement technique for assessing signaling potency (EC_{50}) and/or efficacy (E_{max}). Measurements curated are for pathways that the wild-type receptor doesn't natively signal through, or weakly signals through. Columns 6-7, comparison of signaling potency for the wild-type receptor versus the mutant. Columns 8-9, comparison of signaling efficacy for the wild-type receptor versus the mutant. Column 10, notes on which values are curated and/or additional mutations noted for the receptor which may affect changes in receptor function. Column 11, reference from which data is derived.

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