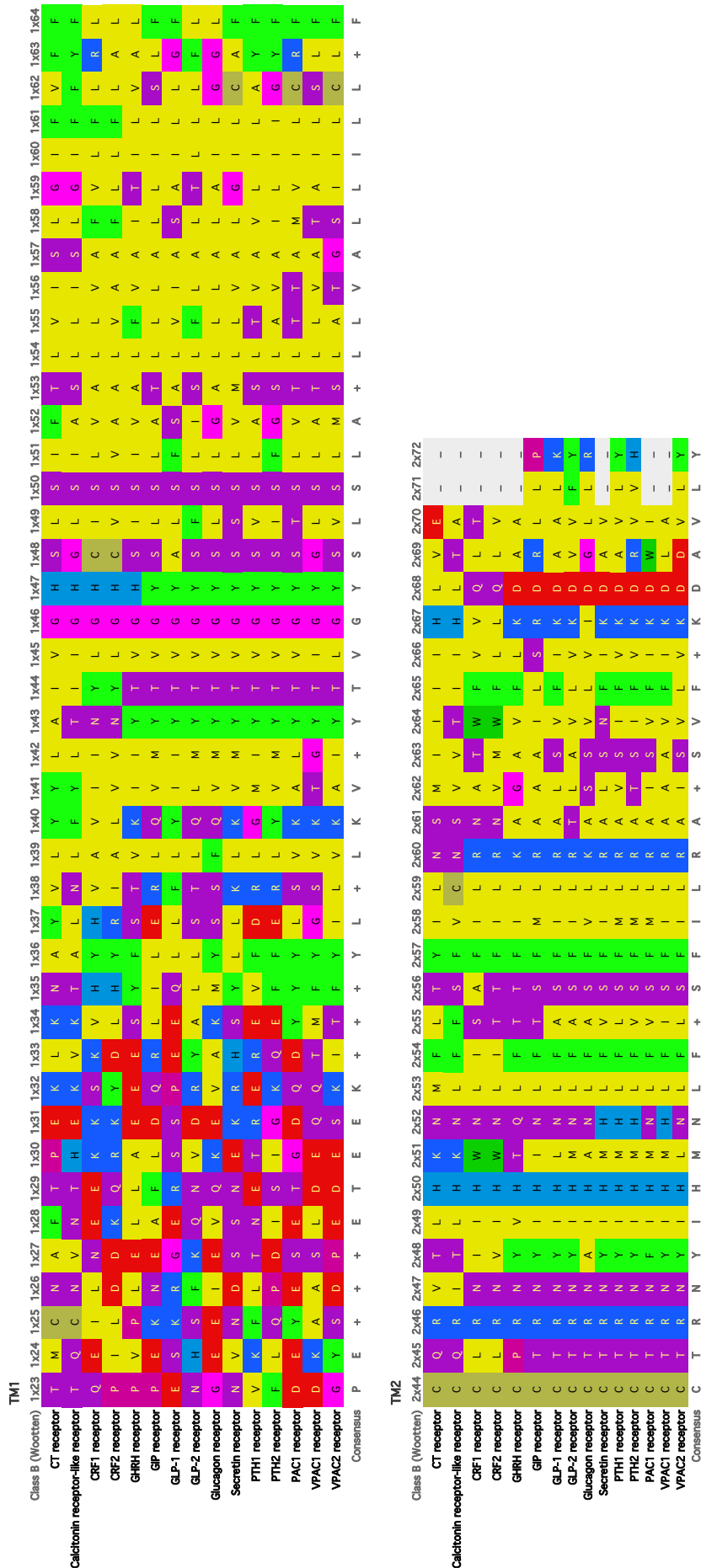


Supplementary Information: Table 1 Refinement and model statistics

Data collection	
Particles	
Pixel size (Å)	1.06
Defocus (μm)	0.5
Voltage (kV)	300
Electron dose (e ⁻ /Å ²)	50
EM map refinement	
Resolution (Å)	3.8
Map sharpened B-factor (Å ²)	-128
Model refinement	
Non-hydrogen atoms	7643
R. M. S. Deviations	
Bond length (Å)	0.007
Bond angle (°)	1.168
Ramachandran plot	
Favored (%)	95.57
Allowed (%)	4.43
Outlier (%)	0
Rotamer outliers (%)	0.25
Validation	
EMRinger Score	1.88
MolProbity score	1.73
All-atom clash score	7.75

Supplementary figure 1

Class B GPCRs TM and H8 sequence alignment. An initial GPCrdB (<http://gpcrdb.org/>) alignment was modified by removing gaps followed by an update of the consensus residues. The Wootten class B numbering system was applied to the alignment. Chemically similar amino acids are noted by equivalent coloured box shading, coloured according to default settings in GPCrdB. The consensus sequence is displayed at the bottom of the alignment.



	3x22	3x23	3x24	3x25	3x26	3x27	3x28	3x29	3x30	3x31	3x32	3x33	3x34	3x35	3x36	3x37	3x38	3x39	3x40	3x41	3x42	3x43	3x44	3x45	3x46	3x47	3x48	3x49	3x50	3x51	3x52	3x53	3x54	3x55	3x56	3x57	3x58	3x59	3x60		
Class B (Wooten)	3x22	3x23	3x24	3x25	3x26	3x27	3x28	3x29	3x30	3x31	3x32	3x33	3x34	3x35	3x36	3x37	3x38	3x39	3x40	3x41	3x42	3x43	3x44	3x45	3x46	3x47	3x48	3x49	3x50	3x51	3x52	3x53	3x54	3x55	3x56	3x57	3x58	3x59	3x60		
CT receptor	-	-	-	-	-	-	V	S	C	K	I	L	H	F	F	I	H	L	Q	Y	M	A	C	N	Y	F	W	M	L	C	E	G	I	Y	L	H	T	L	I	V	V
Calcitonin receptor-like receptor	-	-	-	-	-	-	V	S	C	K	V	S	Q	F	F	I	H	L	Q	Y	M	A	C	N	Y	F	W	M	L	C	E	G	I	Y	L	H	T	L	I	V	V
CRF1 receptor	-	-	-	-	-	-	G	W	C	R	L	V	T	A	A	Y	N	F	H	V	V	T	N	F	F	W	M	F	G	E	G	C	Y	L	H	T	A	I	V	L	
CRF2 receptor	-	-	-	-	-	-	V	W	C	R	C	I	T	T	I	F	N	F	F	V	V	T	N	F	F	W	M	F	G	E	G	C	Y	L	H	T	A	I	V	L	
GHRH receptor	-	-	-	-	-	-	V	L	A	C	K	V	S	V	A	S	H	F	A	T	V	T	N	F	S	W	L	A	E	A	E	G	V	Y	L	N	C	L	L	A	S
GIP receptor	W	N	Q	A	L	A	A	A	C	R	T	A	Q	I	V	T	Q	Y	C	V	G	A	N	Y	Y	W	L	L	V	E	G	V	Y	L	H	S	L	L	A	S	
GLP-1 receptor	S	Y	Q	D	S	L	S	S	C	R	L	V	F	L	L	M	Q	Y	C	V	A	A	N	Y	Y	W	L	L	V	E	G	V	Y	L	H	T	L	L	A	F	
GLP-2 receptor	L	S	E	M	S	T	S	C	R	V	S	Q	Q	V	L	L	H	Q	Y	C	V	A	A	N	Y	Y	W	L	L	V	E	G	L	Y	L	H	T	L	L	E	P
Glucagon receptor	S	D	G	A	V	A	G	C	R	V	A	A	V	A	V	F	M	Q	Y	G	I	V	A	A	N	Y	C	W	L	L	V	E	G	L	Y	L	H	N	L	L	G
Secretin receptor	-	-	-	-	-	-	A	G	C	K	L	V	M	V	L	F	Q	Y	C	F	I	M	A	N	Y	S	W	L	L	V	E	G	L	Y	L	H	T	L	L	A	F
PTH1 receptor	A	A	A	G	Y	A	G	C	K	L	V	M	V	V	L	F	Q	Y	C	F	L	A	T	N	Y	Y	W	L	L	V	E	G	L	Y	L	H	T	L	L	A	M
PTH2 receptor	D	K	S	Q	Y	I	V	E	C	K	I	A	V	V	M	F	F	I	Y	C	L	A	T	N	Y	Y	W	L	L	V	E	G	L	Y	L	H	T	L	L	A	F
PAC1 receptor	-	-	-	-	-	-	E	E	C	K	A	V	M	V	V	F	F	I	Y	C	V	A	T	N	Y	Y	W	L	L	V	E	G	L	Y	L	H	T	L	L	A	V
VPAC1 receptor	-	-	-	-	-	-	V	G	C	K	A	V	M	V	V	F	F	Q	Y	C	V	M	A	N	Y	F	W	L	L	V	E	G	L	Y	L	H	T	L	L	A	V
VPAC2 receptor	Q	P	S	S	W	V	G	C	K	L	A	S	L	V	F	F	Q	Y	C	I	M	A	N	Y	F	W	L	L	V	E	G	L	Y	L	H	T	L	L	A	V	
Consensus	S	+	+	A	+	V	G	C	K	+	S	+	+	V	F	F	F	Q	Y	C	V	+	A	N	Y	F	W	L	L	V	E	G	L	L	H	T	L	L	A	V	

	4x40	4x41	4x42	4x43	4x44	4x45	4x46	4x47	4x48	4x49	4x50	4x51	4x52	4x53	4x54	4x55	4x56	4x57	4x58	4x59	4x60	4x61	4x62	4x63	4x64	4x65	4x66	4x67	4x68
Class B (Wooten)	Q	R	L	R	W	Y	Y	L	L	G	W	G	F	P	L	V	P	T	I	I	H	A	I	T	R	A	V	Y	F
CT receptor	Q	H	L	M	W	Y	Y	F	L	G	W	G	F	P	L	I	P	A	C	I	H	A	I	A	R	A	L	Y	F
Calcitonin receptor-like receptor	L	R	K	W	M	F	C	I	C	I	G	W	G	V	F	I	I	V	A	A	A	A	I	G	K	L	Y	Y	D
CRF1 receptor	L	R	K	C	L	F	F	L	F	I	G	W	C	I	P	F	P	I	V	A	W	A	I	G	K	L	Y	Y	E
CRF2 receptor	R	A	F	W	W	L	V	L	A	G	W	G	L	P	V	L	F	T	G	I	W	V	V	S	C	K	L	A	F
GHRH receptor	G	H	F	R	Y	Y	L	L	L	L	G	W	G	A	L	F	L	F	I	P	W	W	V	I	V	V	L	Y	E
GIP receptor	W	I	F	R	L	Y	V	S	I	G	W	G	G	V	P	L	F	V	V	P	W	G	I	V	V	K	Y	L	E
GLP-1 receptor	R	L	W	P	R	L	L	L	L	G	W	A	F	P	L	F	V	V	P	P	W	A	V	V	K	C	L	F	E
GLP-2 receptor	S	F	S	L	Y	L	L	G	I	L	G	W	A	F	P	M	L	F	V	P	W	A	V	V	K	C	L	F	E
Glucagon receptor	K	Y	L	Q	G	F	T	A	F	G	W	G	S	P	A	I	F	V	A	L	W	A	V	V	S	A	R	H	E
Secretin receptor	K	Y	L	W	W	F	V	V	L	G	W	G	L	G	A	V	F	V	A	V	W	A	V	V	S	A	R	L	E
PTH1r receptor	K	Y	L	W	G	F	I	L	I	G	W	G	G	F	P	A	I	F	V	A	W	A	V	A	V	A	T	L	A
PTH2 receptor	K	Y	F	W	G	F	I	L	I	G	W	G	G	F	P	A	F	V	A	A	W	A	V	A	V	A	T	L	A
PAC1 receptor	K	Y	F	W	G	F	I	L	I	G	W	G	T	P	T	C	V	A	V	W	W	A	T	T	L	Y	F	D	
VPAC1 receptor	K	Y	F	W	G	F	I	L	I	G	W	G	V	P	S	T	F	M	V	W	W	T	I	A	R	I	H	F	E
VPAC2 receptor	R	C	F	W	L	A	Y	L	L	I	G	W	G	L	P	T	V	C	I	W	A	A	T	A	A	R	L	Y	E
Consensus	+	Y	F	W	+	Y	L	L	L	I	G	W	G	F	P	L	F	V	+	W	A	I	A	R	L	+	Y	E	D

Class B (Wootton)		5x32	5x33	5x34	5x35	5x36	5x37	5x38	5x39	5x40	5x41	5x42	5x43	5x44	5x45	5x46	5x47	5x48	5x49	5x50	5x51	5x52	5x53	5x54	5x55	5x56	5x57	5x58	5x59	5x60	5x61	5x62	5x63	5x64	5x65	5x66	5x67	
CT receptor	E	T	H	L	L	L	Y	I	I	H	G	P	V	M	A	A	L	V	V	N	F	F	F	L	L	N	I	V	R	V	V	L	V	I	K	M	-	
Calcitonin receptor-like receptor	D	T	H	L	L	L	Y	I	I	H	G	P	V	M	A	A	L	L	L	V	N	F	F	F	L	L	N	I	V	R	V	L	V	I	K	L	-	
CRF1 receptor	-	-	-	T	D	Y	Y	Y	Y	Q	G	P	M	I	L	V	L	L	L	N	F	F	F	L	F	N	I	V	R	R	L	L	M	T	K	L	-	
CRF2 receptor	-	-	-	-	V	D	Y	Y	Y	Q	G	P	M	I	L	V	L	L	L	N	F	F	F	L	F	N	I	V	R	R	L	L	M	T	K	L	-	
GHRH receptor	T	S	P	Y	W	W	I	I	K	R	T	P	V	L	V	S	V	G	V	N	F	G	L	F	L	N	I	V	R	R	L	L	V	R	K	L	-	
GIP receptor	Y	S	A	I	W	W	I	I	K	R	T	P	V	L	M	T	V	G	V	N	F	F	L	F	L	N	I	V	R	R	L	L	V	I	K	L	-	
GLP-1 receptor	N	M	N	Y	W	L	I	I	R	L	L	P	I	L	F	A	I	G	V	N	F	F	L	F	L	N	I	V	R	R	V	L	V	I	S	K	L	-
GLP-2 receptor	N	K	I	W	W	W	I	I	R	G	P	M	I	L	C	V	T	G	V	N	F	F	F	L	F	N	I	V	R	R	L	L	V	S	K	L	-	
Glucagon receptor	N	M	G	F	W	W	I	L	R	F	P	M	I	L	F	L	T	G	V	N	F	F	F	L	F	N	I	V	R	R	L	L	V	S	K	L	-	
Secretin receptor	N	A	S	I	W	W	I	I	R	G	P	V	L	L	S	I	L	V	L	N	F	F	L	F	L	N	I	V	R	R	V	L	L	M	R	K	L	-
PTH1 receptor	S	G	N	K	W	W	I	I	Q	V	P	P	L	L	A	S	I	V	L	N	F	F	L	F	L	N	I	V	R	R	V	L	L	A	T	K	L	-
PTH2 receptor	Y	A	D	I	K	W	I	Y	Q	A	P	P	V	L	A	A	I	G	V	N	F	F	L	F	L	N	I	V	R	R	V	L	L	A	T	K	L	-
PAC1 receptor	S	T	A	L	W	W	V	V	K	G	P	P	V	L	G	S	I	M	V	N	F	F	L	F	L	N	I	V	R	R	V	L	L	V	Q	K	L	-
VPAC1 receptor	N	S	L	W	W	W	I	I	K	G	P	P	V	L	T	S	I	L	V	N	F	F	L	F	L	N	I	V	R	R	L	L	L	Q	K	L	-	
VPAC2 receptor	H	S	V	P	W	W	V	I	R	G	P	P	I	L	T	S	I	L	V	N	F	F	L	F	L	N	I	V	R	R	L	L	V	T	K	L	-	
Consensus	N	S	+	+	W	W	I	I	R	G	P	P	I	L	L	S	I	L	V	N	F	F	L	F	L	N	I	V	R	R	L	L	V	T	K	L	+	A

TM6		6x30	6x31	6x32	6x33	6x34	6x35	6x36	6x37	6x38	6x39	6x40	6x41	6x42	6x43	6x44	6x45	6x46	6x47	6x48	6x49	6x50	6x51	6x52	6x53	6x54	6x55	6x56	6x57	6x58	
Class B (Wootten)	CT receptor	A	E	S	H	M	Y	L	K	A	A	V	K	A	T	M	I	L	V	P	L	L	G	I	Q	F	V	V	F	P	W
Calcitonin receptor-like receptor	CT receptor	A	E	S	N	L	Y	M	K	A	A	V	K	A	T	L	I	L	V	P	L	L	G	I	E	F	V	L	I	P	W
	CRF1 receptor	S	E	T	I	Q	Y	R	K	A	V	K	A	T	L	V	L	L	L	P	L	L	G	I	T	Y	M	L	F	F	V
	CRF2 receptor	S	E	T	J	Q	Y	R	K	A	V	K	A	T	L	V	L	L	L	P	L	L	G	I	T	Y	M	L	F	F	V
	GHRH receptor	H	T	Q	S	Q	Y	W	L	R	L	S	K	S	T	L	L	L	L	P	L	L	G	I	H	Y	I	I	F	N	F
	GLP receptor	-	-	-	-	Y	C	W	R	L	A	R	S	T	L	T	T	L	V	P	L	L	G	I	H	Y	I	I	F	A	P
Glucagon receptor	GLP-1 receptor	-	-	-	-	Y	C	W	R	L	A	K	S	T	L	T	L	L	L	P	L	L	G	I	H	Y	I	I	F	A	P
	GLP-2 receptor	-	-	-	-	Y	C	W	R	L	A	K	S	T	L	V	L	L	L	P	L	L	G	I	H	Y	I	I	F	A	P
	Secretin receptor	N	E	V	S	H	Y	K	R	L	A	K	S	T	L	T	L	L	L	P	L	L	G	I	H	Y	I	I	F	A	P
	PTH1 receptor	D	T	R	Q	Q	Y	R	K	L	L	A	K	S	T	L	L	L	L	P	L	L	G	I	H	Y	I	I	F	A	P
	PTH2 receptor	D	T	R	Q	Q	Y	R	K	L	L	A	K	S	T	L	L	L	L	P	L	L	G	I	H	Y	I	I	F	A	P
VPAC1 receptor	PAC1 receptor	N	E	S	S	J	Y	L	R	L	A	K	S	T	L	L	L	L	L	P	L	L	G	I	H	Y	I	I	F	A	P
	VPAC1 receptor	S	D	S	S	P	Y	S	R	L	A	K	S	T	L	L	L	L	L	P	L	L	G	I	H	Y	I	I	F	A	P
	VPAC2 receptor	N	D	Q	S	Q	Y	K	R	L	A	K	S	T	L	L	L	L	L	P	L	L	G	I	H	Y	I	I	F	A	P
Consensus	+	E	S	S	Q	Y	R	R	L	A	K	S	T	L	V	L	L	L	L	L	L	L	G	+	H	Y	I	V	F	A	F

TM7		7x31	7x32	7x33	7x34	7x35	7x36	7x37	7x38	7x39	7x40	7x41	7x42	7x43	7x44	7x45	7x46	7x47	7x48	7x49	7x50	7x51	7x52	7x53	7x54	7x55	7x56	7x57	7x58	7x59	7x60	
Class B (Wootten)		-	-	-	L	G	K	I	Y	D	Y	V	I	M	S	L	I	H	F	Q	G	F	F	V	A	T	I	Y	C	F	L	
CT receptor		-	-	-	A	E	R	V	Y	D	Y	I	M	H	S	L	I	H	F	Q	G	L	L	V	S	T	I	Y	C	F	L	
Calcitonin receptor-like receptor		-	-	-	V	S	R	V	F	I	Y	F	N	S	F	L	E	S	F	Q	G	F	F	V	S	V	F	Y	C	F	L	
CRF1 receptor		-	-	-	L	S	Q	I	M	F	I	Y	F	N	S	F	L	Q	S	F	Q	G	F	F	V	S	V	Y	C	F	L	
CRF2 receptor		-	-	-	L	S	Q	I	M	F	I	Y	F	N	S	F	L	Q	S	F	Q	G	F	F	V	S	V	Y	C	F	L	
GHRH receptor		-	-	-	-	-	L	G	I	R	L	P	L	E	L	G	S	F	Q	G	G	F	F	I	V	A	I	L	Y	C	F	L
GIP receptor		R	G	A	L	R	F	A	K	L	G	F	E	L	F	L	S	S	F	Q	G	L	M	V	S	V	L	Y	C	F	L	
GLP-1 receptor		R	G	T	L	R	F	E	L	K	L	G	F	E	L	F	S	T	S	F	Q	G	L	M	V	A	I	L	Y	C	F	L
GLP-2 receptor		E	G	F	A	K	L	I	R	L	F	I	Q	L	T	L	S	S	F	H	G	L	L	V	A	L	Y	C	F	L		
Glucagon receptor		Q	G	T	L	L	S	A	K	L	F	F	D	L	F	L	S	S	F	Q	G	L	L	V	A	V	L	Y	C	F	L	
Secretin receptor		-	-	-	-	-	M	E	I	Q	L	F	F	E	L	A	L	G	S	F	Q	G	L	V	V	A	V	L	Y	C	F	L
PTH1 receptor		S	G	T	L	W	Q	V	Q	M	H	Y	E	L	M	L	F	N	S	F	Q	G	F	F	V	A	I	Y	C	F	L	
PTH2 receptor		T	G	L	G	W	E	I	R	M	H	C	E	L	F	F	N	S	F	Q	G	F	F	V	S	I	Y	C	F	L		
PAC1 receptor		-	-	-	-	K	R	E	R	L	V	F	E	L	G	L	G	S	F	Q	G	F	F	V	A	V	L	Y	C	F	L	
VPAC1 receptor		-	-	-	-	P	E	V	R	M	V	F	E	L	V	V	G	S	F	Q	G	F	F	V	V	A	I	L	Y	C	F	L
VPAC2 receptor		-	-	-	-	S	K	Y	Q	I	L	F	E	L	C	L	G	S	F	Q	G	L	F	V	V	A	V	L	Y	C	F	L
Consensus		R	G	T	L	R	E	I	+	L	+	F	E	L	F	L	G	S	F	Q	G	F	F	V	A	V	L	Y	C	F	L	

H8

Class B (Wootten)	8x39	8x40	8x41	8x42	8x43	8x44	8x45	8x46	8x47	8x48	8x49	8x50	8x51	8x52	8x53	8x54	8x55	8x56	8x57	8x58	8x59	8x60	8x61	8x62	8x63	8x64
CT receptor	N	N	E	V	Q	T	T	V	K	R	Q	W	A	Q	F	K	I	Q	W	N	Q	R	W	G	R	R
Calcitonin receptor-like receptor	N	G	E	V	Q	A	I	L	R	R	N	W	N	Q	Y	K	I	Q	F	N	S	I	R	A	R	A
CRF1 receptor	N	S	E	V	R	S	A	I	R	K	R	W	H	R	W	Q	D	K	H	S	L	R	A	P	M	A
CRF2 receptor	N	Q	E	V	R	S	A	I	R	K	R	W	H	R	W	Q	D	K	H	S	L	R	A	P	M	A
GHRH receptor	N	K	E	V	Q	S	E	I	S	R	K	W	H	H	C	H	D	P	E	L	L	P	A	W	T	R
GLP-1 receptor	N	K	E	V	Q	S	E	I	S	R	K	W	H	H	C	H	D	P	E	L	L	P	A	W	T	R
GLP-2 receptor	N	G	E	V	Q	L	E	F	R	K	Y	W	E	R	W	R	L	A	R	H	S	G	R	D	S	C
Glucagon receptor	N	K	E	V	Q	S	E	L	R	R	R	W	H	R	W	R	L	G	K	V	L	W	E	R	N	C
Secretin receptor	N	G	E	V	Q	L	E	F	R	K	Y	W	E	R	W	R	L	A	R	H	S	G	R	D	S	C
PTH1 receptor	N	G	E	V	Q	A	E	I	Q	K	K	W	S	W	Q	W	H	L	A	F	P	L	H	P	V	A
PTH2 receptor	N	G	E	V	Q	A	E	I	Q	K	K	W	S	W	Q	W	H	L	A	F	P	L	H	P	V	A
PAC1 receptor	N	G	E	V	Q	A	E	I	K	R	K	W	S	W	Q	W	H	L	A	F	P	L	H	P	V	A
VPAC1 receptor	N	G	E	V	Q	A	E	I	K	R	K	W	S	W	Q	W	H	L	A	F	P	L	H	P	V	A
VPAC2 receptor	N	S	E	V	Q	C	E	L	K	R	K	W	S	W	Q	W	H	L	A	F	P	L	H	P	V	A
Consensus	N	G	E	V	Q	A	E	+	R	R	K	W	H	R	W	C	P	L	Q	+	S	L	+	R	+	R