Structure of human $GABA_B$ receptor in an inactive state

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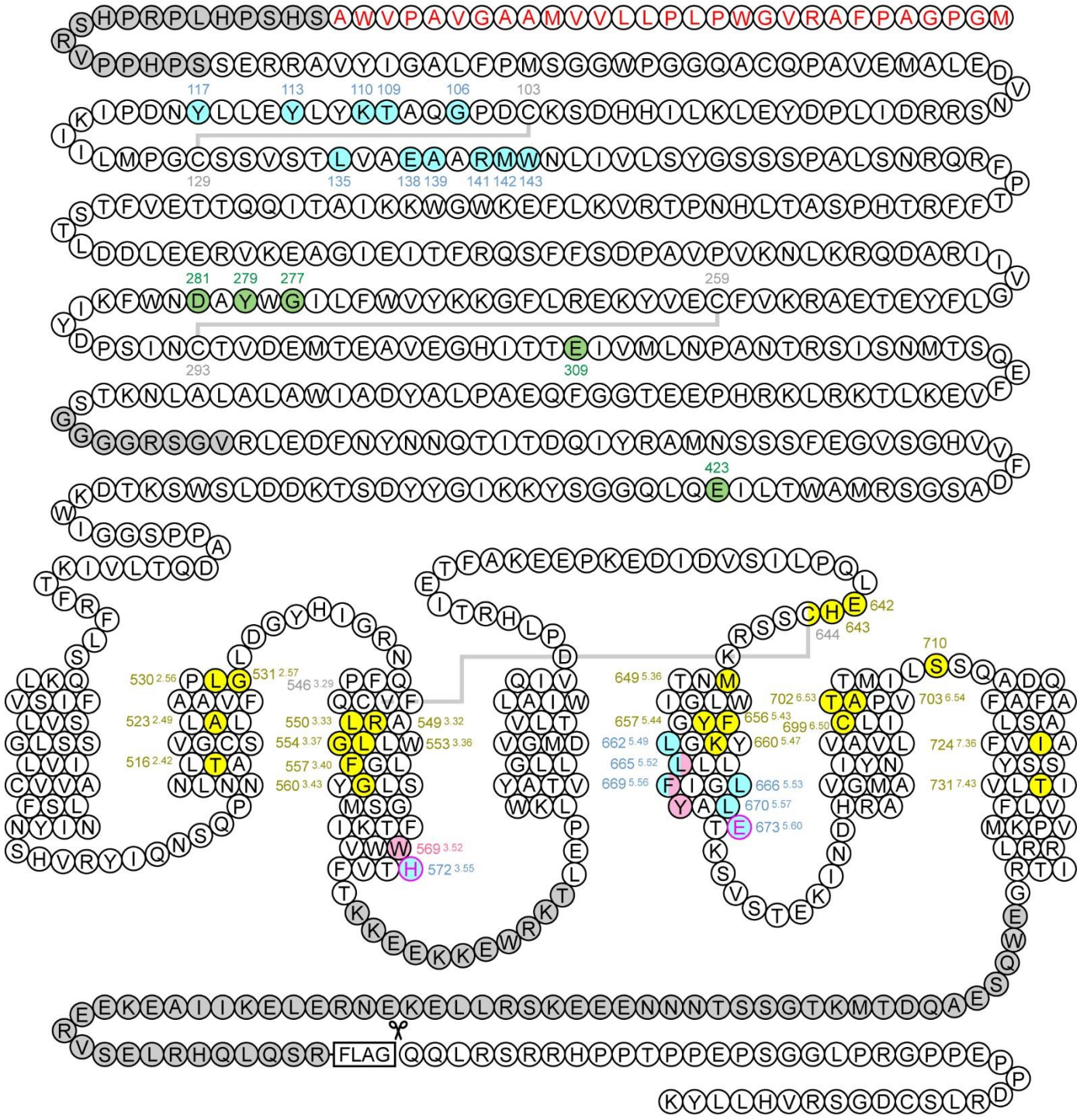
a



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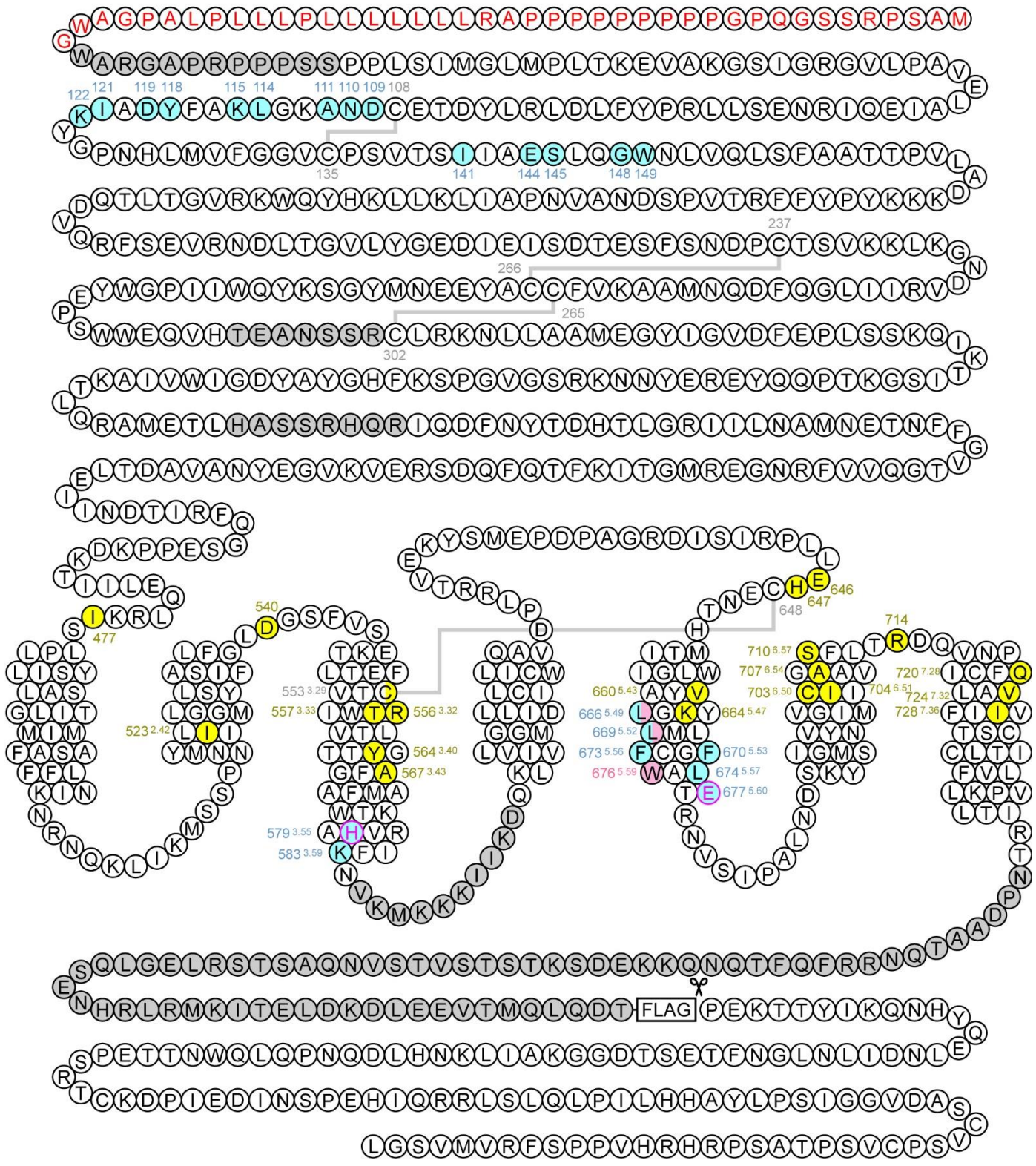
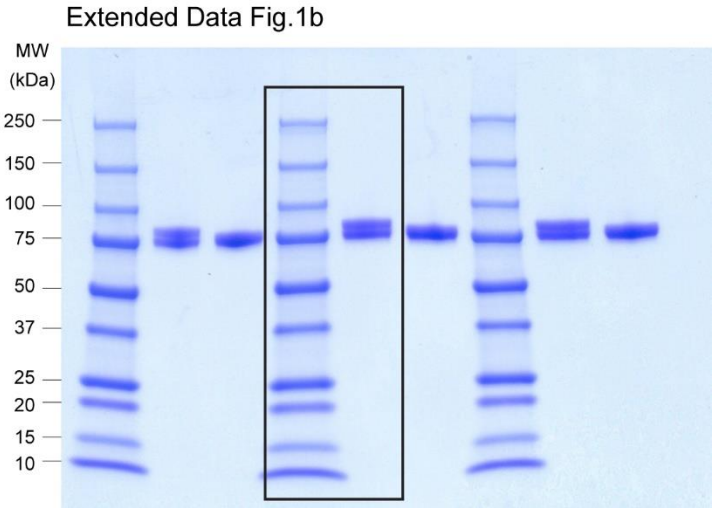
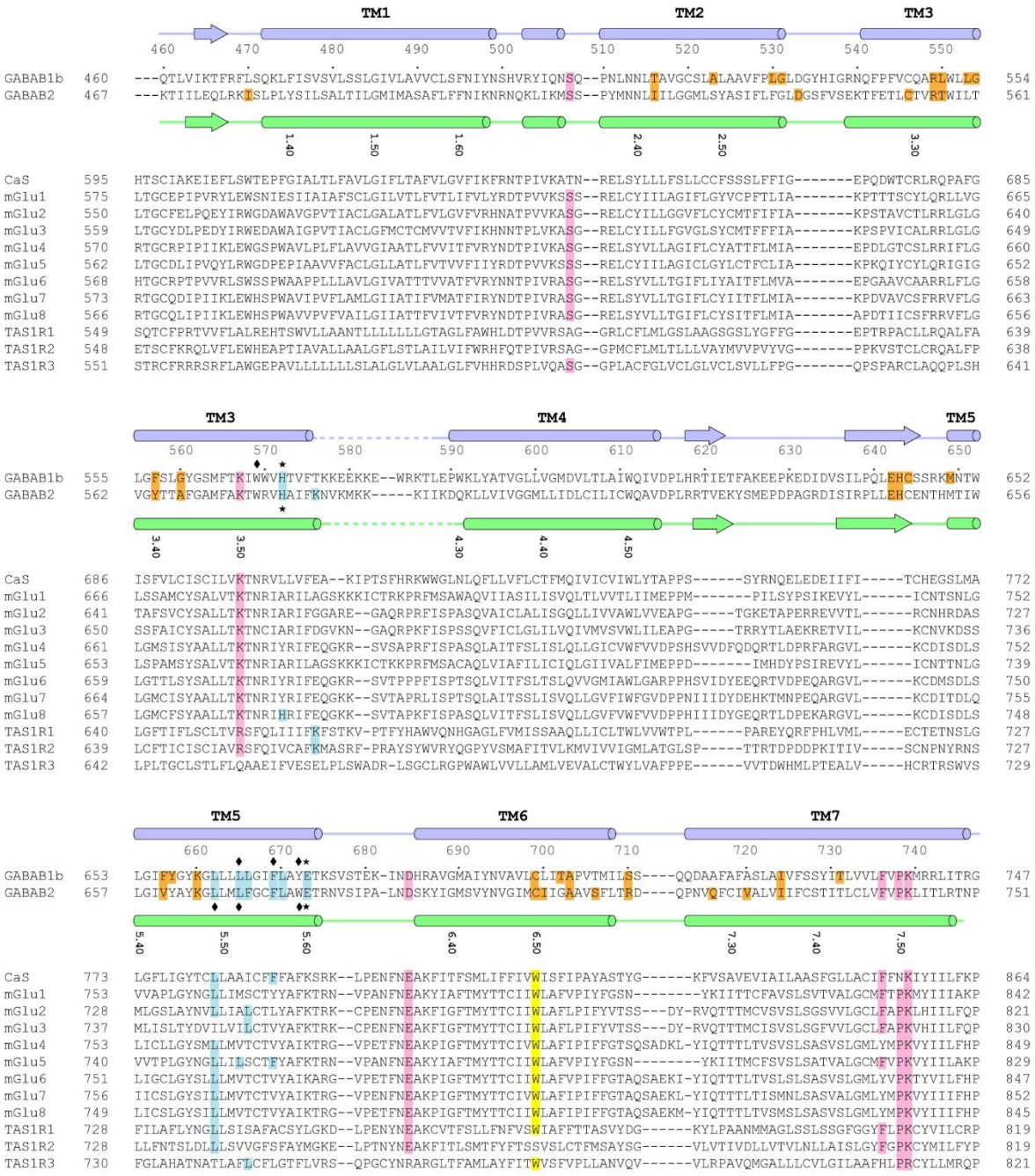


Supplementary Fig. 1 | Schematic diagram of $GABA_B$ receptor subunits.
a,b, Protein sequences of human $GABA_{B1b}$ (**a**) and $GABA_{B2}$ (**b**) depicting key residues in each subunit. The signal peptide of each subunit is in red lettering. The expression construct terminates with a Flag tag, and the remaining residues that are excluded appear after black scissors. Disordered residues in the structure are shaded gray. Disulfide-bonded cysteines are partnered through a gray-colored bold line. Cyan residues form direct contacts at the heterodimeric interface between $GABA_{B1b}$ and $GABA_{B2}$ subunits, while pink residues mediate heterodimer interaction through a cholesterol molecule. Cyan residues with pink lettering and outlines form the ′intersubunit latch′ with each other ($GABA_{B1b}$: $His572^{3.55}$ and $Glu673^{5.60}$; $GABA_{B2}$: $His579^{3.55}$ and $Glu677^{5.60}$). Yellow residues interact with the phospholipid ($GABA_{B1b}$, PE 38:5; $GABA_{B2}$, PC 38:2) within the TM pocket. In the $GABA_{B1b}$ subunit, residues that sequester Ca^{2+} are green.



Supplementary Fig. 2 | Gel source data for Extended Data Fig. 1b.
The content of Extended Data Fig. 1b is highlighted in a box. The experiment was repeated three times with similar results.



Supplementary Fig. 3 | Sequence alignment of human class C GPCRs.
The alignment covers the linker and TM domain of $GABA_B$ receptor and corresponding regions in other class C GPCRs. Secondary structure elements are displayed for $GABA_{B1b}$ (blue) and $GABA_{B2}$ (green) subunits; β-strands are represented by arrows and α-helices by cylinders. Disordered regions are denoted by dashed lines. Alignment of TM helices is based on a modified Ballesteros-Weinstein numbering system for class C receptors, with TM positions marked for every ten residues around X.50 (X refers to TM helix number)[59,60]. $GABA_B$ residues involved in phospholipid binding are highlighted in orange. Contacting residues at the heterodimer interface between $GABA_{B1b}$ and $GABA_{B2}$ subunits are highlighted in cyan; diamonds indicate residues that mediate heterodimeric interaction through cholesterol; stars mark residues that form the ′intersubunit latch′ ($GABA_{B1b}$: $His572^{3.55}$ and $Glu673^{5.60}$; $GABA_{B2}$: $His579^{3.55}$ and $Glu677^{5.60}$). For all receptors, heterodimer interface residues of $GABA_B$ that are conserved in other class C receptors are also in cyan, similarly charged or wholly conserved residues that correspond to the intramolecular ′ionic lock′ and FxPKxx motifs of $GABA_B$ receptor are in pink, conserved tryptophan of the toggle motif is in yellow.

Supplementary Table 1 | Cryo-EM data collection, refinement and validation statistics

	Human $GABA_B$ receptor (EMD-21685) (PDB 6WIV)
Data collection and processing	
Magnification	130,000 x
Voltage (kV)	300
Electron exposure (e–/Å^2)	85
Defocus range (μm)	-0.5 to -2
Pixel size (Å)	1.06
Symmetry imposed	C1
Initial particle images (no.)	1,048,241
Final particle images (no.)	233,737
Map resolution (Å)	3.1 (ECD); 3.4 (TM); 3.3 (Global)
FSC threshold	0.143
Map resolution range[1] (Å)	2.8-7.3 (ECD); 2.9-9.3 (TM); 3.0-6.4 (Global)
Refinement	
Initial model used (PDB code)	4MQE
Model resolution (Å)	3.3
FSC threshold	0.5
Model resolution range[2] (Å)	3.0-6.0
Map sharpening *B* factor (Å^2)	-75.0 (ECD); -79.0 (TM); -91.2 (Global)
Model composition	
Non-hydrogen atoms	11,213
Protein residues	1,352
Ligands - Ca^{2+}	1
Sugar	4
Cholesterol	10
Phospholipid	2
B factors (Å^2)	
Protein	69.0
Ligands - Ca^{2+}	71.6
Sugar	89.8
Cholesterol	67.0
Phospholipid	61.2
R.m.s. deviations	
Bond lengths (Å)	0.005
Bond angles (°)	0.71
Validation	
MolProbity score	1.49
Clashscore	5.49
Poor rotamers (%)	0.08
Ramachandran plot	
Favored (%)	96.9
Allowed (%)	3.1
Disallowed (%)	0.0

[1]Total range of local resolution values within the mask used to calculate FSC.
[2]Range of local resolution values at atom positions, averaged per residue.

Supplementary Table 2 | Transmembrane helices in $GABA_B$ receptor subunits

TM1			TM2			TM3			TM4			TM5			TM6			TM7		
	B1b	B2		B1b	B2		B1b	B2		B1b	B2		B1b	B2		B1b	B2		B1b	B2
1.37	Q472	L479	2.36	P510	P517	3.22	-	E546	4.29	P590	-	5.36	M649	M653	6.36	H685	S689	7.25	Q713	P717
1.38	K473	P480	2.37	N511	Y518	3.23	-	K547	4.30	W591	-	5.37	N650	T654	6.37	R686	K690	7.26	D714	N718
1.39	L474	L481	2.38	L512	M519	3.24	Q541	T548	4.31	K592	K596	5.38	T651	I655	6.38	A687	Y691	7.27	A715	V719
1.40	F475	Y482	2.39	N513	N520	3.25	F542	F549	4.32	L593	L597	5.39	W652	W656	6.39	V688	I692	7.28	A716	Q720
1.41	I476	S483	2.40	N514	N521	3.26	P543	E550	4.33	Y594	L598	5.40	L653	L657	6.40	G689	G693	7.29	F717	F721
1.42	S477	I484	2.41	L515	L522	3.27	F544	T551	4.34	A595	V599	5.41	G654	G658	6.41	M690	M694	7.30	A718	C722
1.43	V478	L485	2.42	T516	I523	3.28	V545	L552	4.35	T596	I600	5.42	I655	I659	6.42	A691	S695	7.31	F719	I723
1.44	S479	S486	2.43	A517	I524	3.29	C546	C553	4.36	V597	V601	5.43	F656	V660	6.43	I692	V696	7.32	A720	V724
1.45	V480	A487	2.44	V518	L525	3.30	Q547	T554	4.37	G598	G602	5.44	Y657	Y661	6.44	Y693	Y697	7.33	S721	A725
1.46	L481	L488	2.45	G519	G526	3.31	A548	V555	4.38	L599	G603	5.45	G658	A662	6.45	N694	N698	7.34	L722	L726
1.47	S482	T489	2.46	C520	G527	3.32	R549	R556	4.39	L600	M604	5.46	Y659	Y663	6.46	V695	V699	7.35	A723	V727
1.48	S483	I490	2.47	S521	M528	3.33	L550	T557	4.40	V601	L605	5.47	K660	K664	6.47	A696	G700	7.36	I724	I728
1.49	L484	L491	2.48	L522	L529	3.34	W551	W558	4.41	G602	L606	5.48	G661	G665	6.48	V697	I701	7.37	V725	I729
1.50	G485	G492	2.49	A523	S530	3.35	L552	I559	4.42	M603	I607	5.49	L662	L666	6.49	L698	M702	7.38	F726	F730
1.51	I486	M493	2.50	L524	Y531	3.36	L553	L560	4.43	D604	D608	5.50	L663	L667	6.50	C699	C703	7.39	S727	C731
1.52	V487	I494	2.51	A525	A532	3.37	G554	T561	4.44	V605	L609	5.51	L664	M668	6.51	L700	I704	7.40	S728	S732
1.53	L488	M495	2.52	A526	S533	3.38	L555	V562	4.45	L606	C610	5.52	L665	L669	6.52	I701	I705	7.41	Y729	T733
1.54	A489	A496	2.53	V527	I534	3.39	G556	G563	4.46	T607	I611	5.53	L666	F670	6.53	T702	G706	7.42	I730	I734
1.55	V490	S497	2.54	F528	F535	3.40	F557	Y564	4.47	L608	L612	5.54	G667	G671	6.54	A703	A707	7.43	T731	T735
1.56	V491	A498	2.55	P529	L536	3.41	S558	T565	4.48	A609	I613	5.55	I668	C672	6.55	P704	A708	7.44	L732	L736
1.57	C492	F499	2.56	L530	F537	3.42	L559	T566	4.49	I610	C614	5.56	F669	F673	6.56	V705	V709	7.45	V733	C737
1.58	L493	L500	2.57	G531	G538	3.43	G560	A567	4.50	W611	W615	5.57	L670	L674	6.57	T706	S710	7.46	V734	L738
1.59	S494	F501				3.44	Y561	F568	4.51	Q612	Q616	5.58	A671	A675	6.58	M707	F711	7.47	L735	V739
1.60	F495	F502				3.45	G562	G569	4.52	I613	A617	5.59	Y672	W676	6.59	I708	L712	7.48	F736	F740
1.61	N496	N503				3.46	S563	A570	4.53	V614	V618	5.60	E673	E677				7.49	V737	V741
1.62	I497	I504				3.47	M564	M571				5.61	T674	T678				7.50	P738	P742
1.63	Y498	K505				3.48	F565	F572										7.51	K739	K743
1.64	N499	-				3.49	T566	A573										7.52	M740	L744
						3.50	K567	K574										7.53	R741	I745
						3.51	I568	T575										7.54	R742	T746
						3.52	W569	W576										7.55	L743	L747
						3.53	W570	R577										7.56	I744	R748
						3.54	V571	V578										7.57	T745	-
						3.55	H572	H579										7.58	R746	-
						3.56	T573	A580												
						3.57	V574	I581												
						3.58	F575	F582												
						3.59	-	K583												

Numbering of TM helix residues is based on a modified Ballesteros-Weinstein system for class C receptors (*59*, *60*).

Supplementary Table 3 | Elemental analysis of $GABA_B$ receptor

		Mg	Ca	Mn	Fe	Co	Ni	Cu	Zn	Sr
Buffer [M_B][1]	µg/L	0.09	9.19	0.00	0.00	0.00	0.00	0.02	0.07	0.04
	C.V.[2]	4.7%	3.5%	20.5%	19.3%	43.7%	98.2%	10.6%	4.7%	1.9%
Protein[3] [M_P][1]	µg/L	0.41	28.91	0.01	3.79	0.20	8.23	36.95	1.80	0.17
	C.V.[2]	2.7%	2.0%	3.9%	1.1%	2.5%	2.2%	2.1%	1.4%	1.5%
Net [M][1] ([M_p] - [M_B])	µg/L	0.32	19.72	0.01	3.79	0.20	8.23	36.93	1.73	0.13
	µM	0.01	0.49	0.00	0.07	0.00	0.14	0.58	0.03	0.00
Ratio[4] Net [M] / [P]		0.01	0.43	0.00	0.06	0.00	0.12	0.51	0.02	0.00

[1][M_B] is metal concentration in buffer; [MP] is metal concentration in $GABA_B$ receptor protein sample; Net [M] is the metal concentration in protein sample after buffer correction.
[2]C.V. is coefficient of variance
[3]Protein concentration in $GABA_B$ receptor sample [P] is 1.15 µM.
[4]Molar ratio of net metal concentration to protein concentration.
Data represents average of eight measurements within two experiments.

Supplementary references

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60 Pin, J. P., Galvez, T. & Prezeau, L. Evolution, structure, and activation mechanism of family 3/C G-protein-coupled receptors. *Pharmacol. Ther.* **98**, 325-354, (2003).