

Supplementary Figures

Structural analysis of pathogenic missense mutations in *GABRA2* and identification of a novel *de novo* variant in the desensitization gate

Alba Sanchis-Juan^{1,2,*}, Marcia A Hasenahuer^{3,4,*}, James A Baker³, Amy McTague⁵, Katy Barwick⁵, Manju A Kurian⁵, Sofia T Duarte⁶, NIHR BioResource, Janet Thornton³, F Lucy Raymond^{2,4}

1. Department of Haematology, University of Cambridge, NHS Blood and Transplant Centre, Cambridge CB2 0PT, United Kingdom

2. NIHR BioResource, Cambridge University Hospitals NHS Foundation Trust, Cambridge Biomedical Campus, Cambridge CB2 0QQ, UK

3. European Molecular Biology Laboratory, European Bioinformatics Institute, Wellcome Genome Campus, Hinxton, Cambridge, CB10 1SD, United Kingdom

4. Department of Medical Genetics, Cambridge Institute for Medical Research, University of Cambridge, Cambridge CB2 0XY, UK

5. Developmental Neurosciences, Great Ormond Street Institute of Child Health, University College London, London WC1N 1EH, UK

6. Hospital Dona Estefânia, Centro Hospitalar de Lisboa Central, 1169-045 Lisbon, Portugal

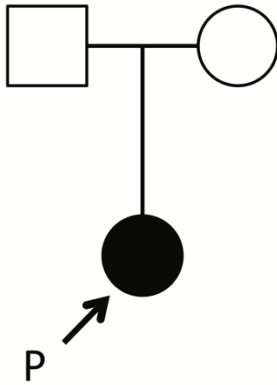
* These authors contributed equally to this work

Corresponding author

Professor F Lucy Raymond, email: flr24@cam.ac.uk

Supplementary Figure 1. Sanger sequencing confirmation of the variant identified in *GABRA2*. A) Family pedigree structure. B) Sanger sequencing confirmation results. The variant was observed to be *de novo* in the proband.

A

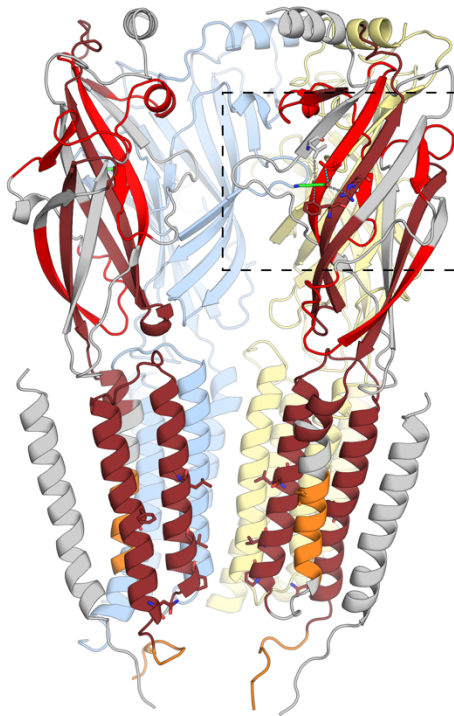


B

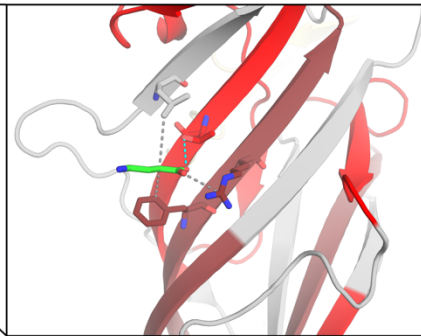
Disease status	<i>GABRA2</i> c.839C>T	Chromatogram
Affected	Yes	
Father	No	
Mother	No	

Supplementary Figure 2. A) Constrained coding regions (CCRs) for *GABRA2* mapped in the GABA_A $\alpha 1\beta 3\gamma 2$ receptor in desensitized state. Colors for the CCRs match the ones used in Figure 1, and the variants highlighting is consistent with symbols as in Figure 1. The $\beta 3$ chain is shown in blue and the $\gamma 2$ is in yellow. **B) Close view of one of the GABA binding sites.** The GABA molecule is depicted in green. PDB ID of the GABA_A $\alpha 1\beta 3\gamma 2$ receptor: 6HUP.

A)

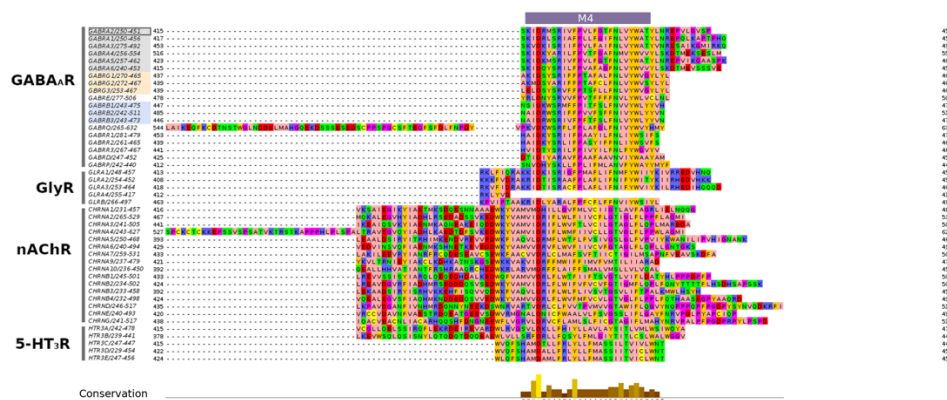
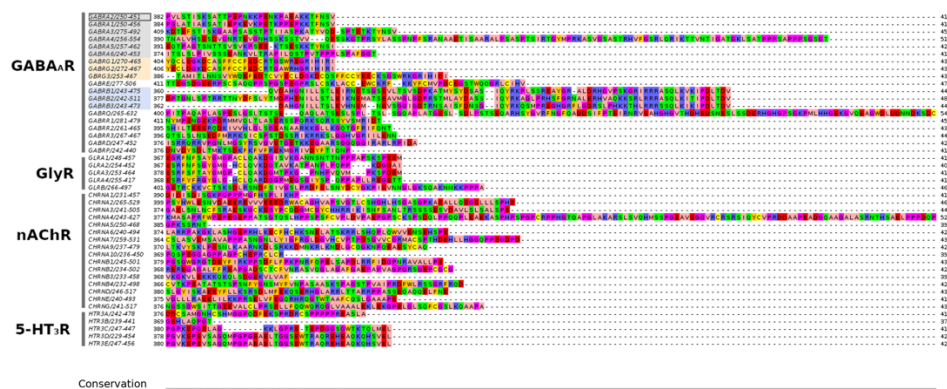
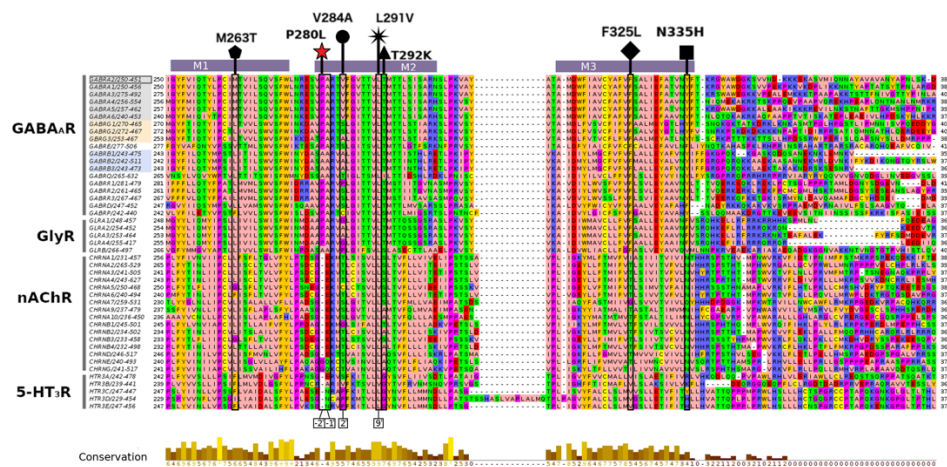


B)

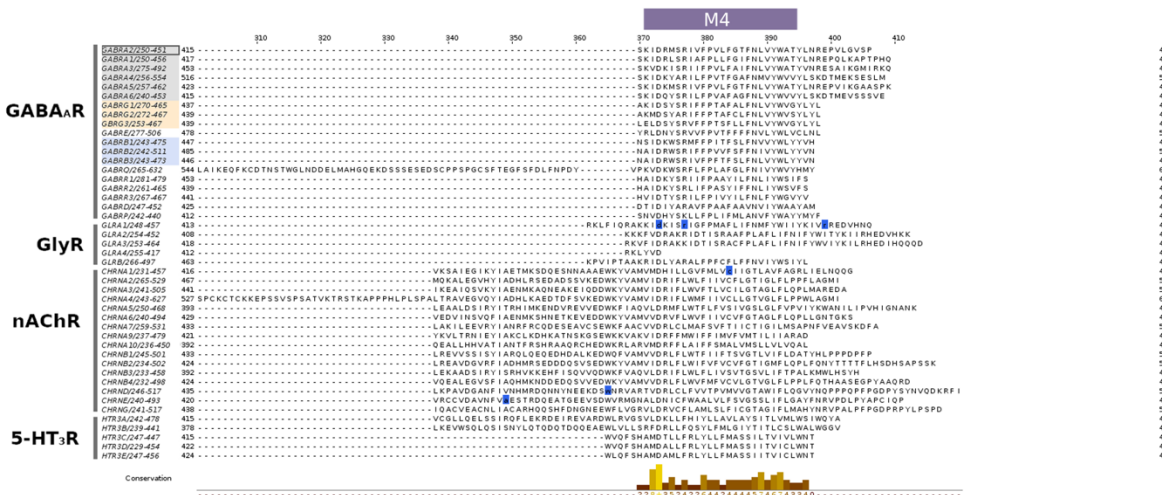
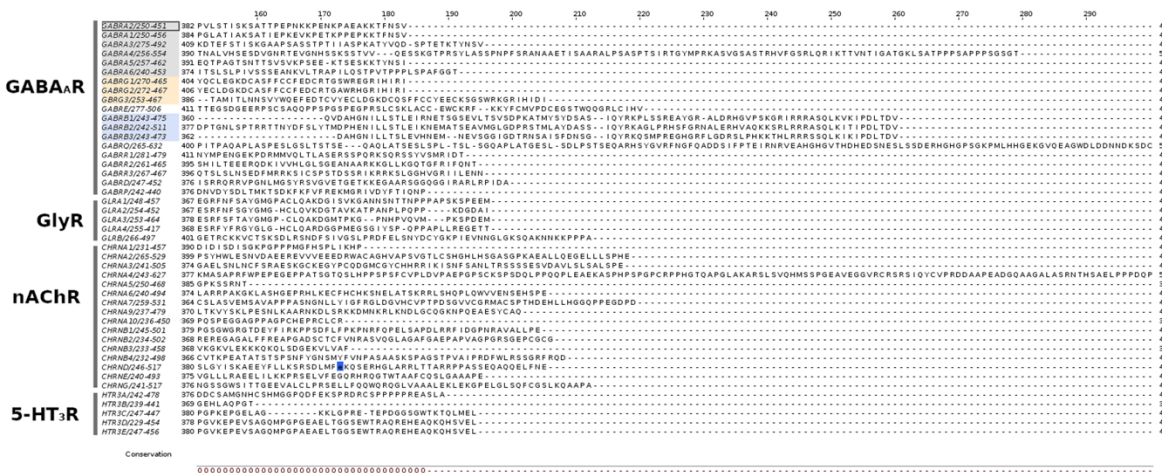
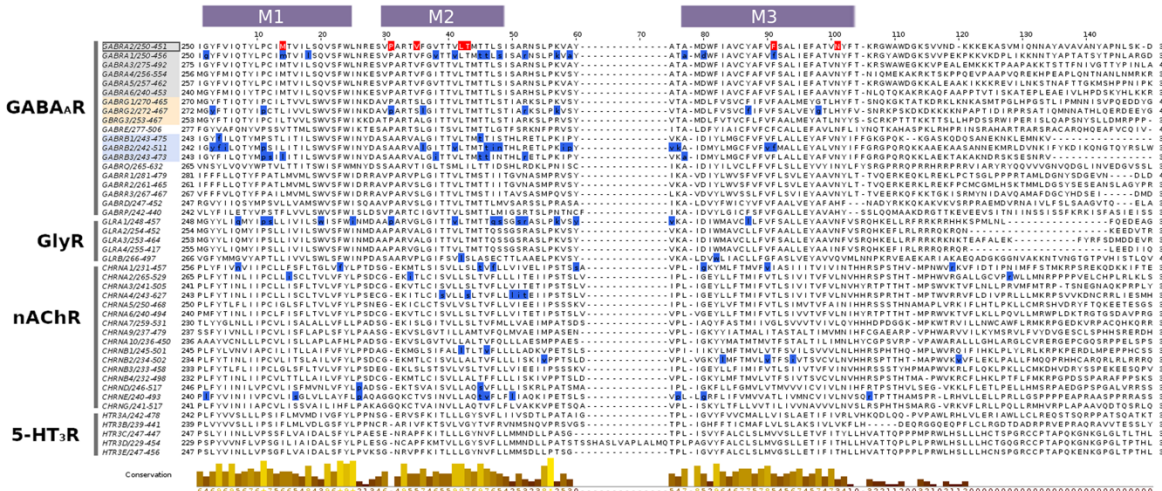


Supplementary Figure 3. Multiple sequence alignment of the TM domain of GABA_A, Gly, nACh and 5-HT₃ members of the Cys-loop receptors from human. The novel and previously reported variants in *GABRA2* are represented in the corresponding columns of the alignment, using the same symbols as in Figure 1. The approximate boundaries of the TMH, depicted with the violet bars, are based on GABA_Aα1R structures (PDB IDs 6HUG, 6HUP and 6I53). **A)** Residues are colored according to their physicochemical properties (Jalview color scheme). **B)** Residues in blue correspond with pathogenic variants in ClinVar and Humsavar. Residues in red correspond with the novel and previously reported variants in *GABRA2*, discussed in this study.

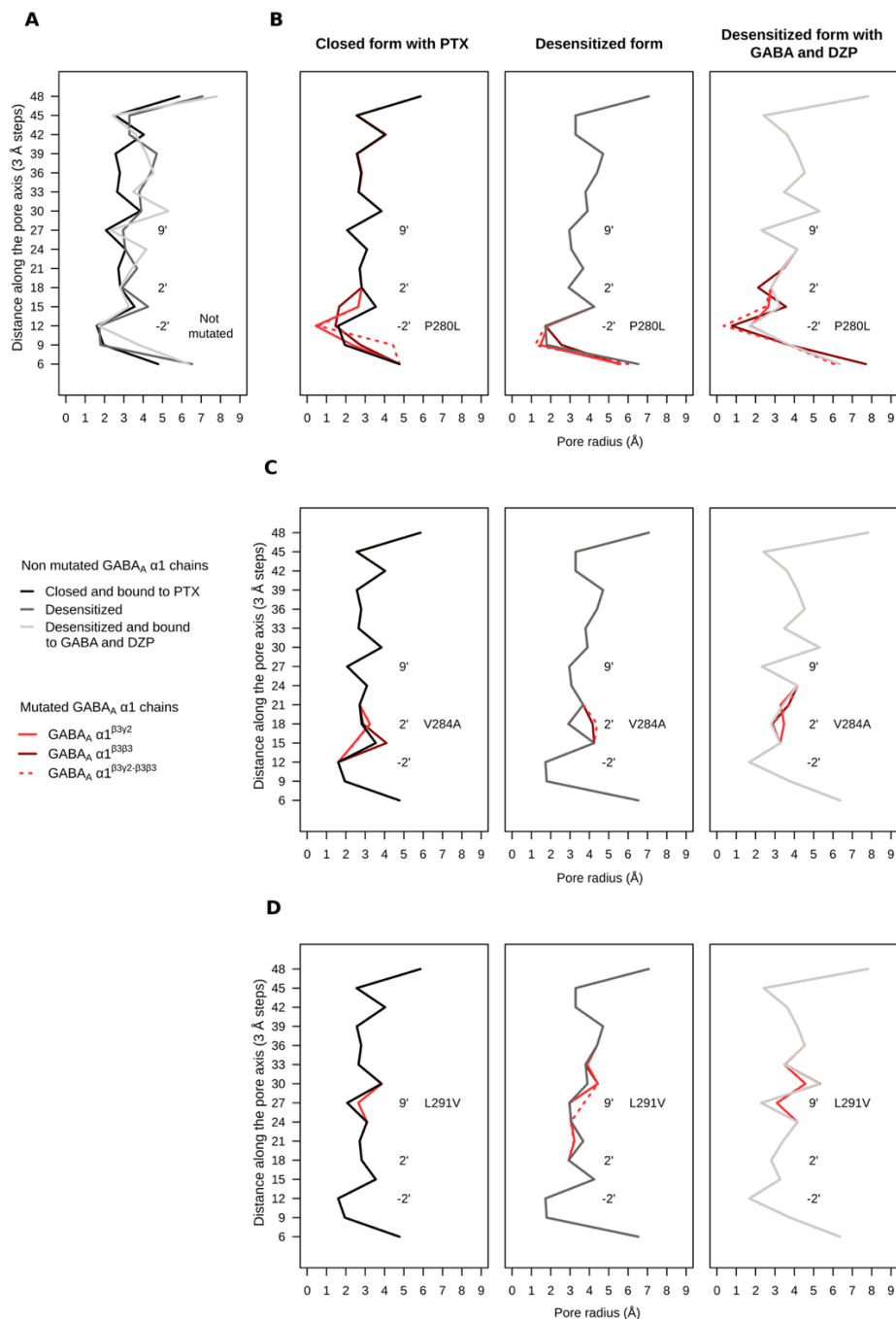
A)



B)



Supplementary Figure 4. Radii profile measured at steps of 3 Å through the TM pore axis in the closed and desensitized states of the hetero-pentameric GABA_A α 1 β 3 γ 2 receptor. The closed structure is in complex with PTX (PDB ID 6HUG). Two desensitized structures have been used, with and without GABA and DZP (PDB ID 6HUP and 6I53 respectively). **(A)** Profiles comparing the non-mutant structures. For the mutant structures, only the pore lining variants have been considered: **(B)** P280L, **(C)** V284A, **(D)** L291V, when the mutant α 1 subunit is between β 3 γ 2 (α 1 β 3 γ 2), between β 3 β 3 (α 1 β 3 β 3), and when both α 1 subunits are mutated (α 1 β 3 γ 2- β 3 β 3). PTX=picROTOXIN, DZP=diazepam.



Supplementary Figure 5. Anaesthetic-binding site of the diazepam (DZP) in the β 3- α 1 interface of the transmembrane (TM) domain of the GABA_A α 1 β 3 γ 2 receptor. This receptor is in complex with DZP and GABA (PDB ID 6HUP). **A)** Side view of the β 3- α 1 interface. Exposed surface is colored according to electrostatic surface potential. **B)** Close view from the top of the cavity where the DZP (yellow) binds. DZP interacts directly with M263 and T292, and is close to the leucines that define the 9' ring. Symbols of the disease causing variants reported in *GABRA2* are the same as in Figure 1.

