Supplemental Material

Analgesic $\alpha$-Conotoxin Binding Site on the Human GABA$_B$ Receptor
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Table S1. Stereochemical evaluation of $\alpha$-Ctx-VFT models using PROCHECK, VERIFY3D, and ProSA protein structure assessment tools.

<table>
<thead>
<tr>
<th>$\alpha$-Ctx-VFT Complex</th>
<th>PROCHECK</th>
<th>VERIFY3D</th>
<th>ProSA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ramachandran plot statistics (%)</td>
<td>Chi1-Chi2 statistics (%)</td>
<td>Compatibility score (%)</td>
</tr>
<tr>
<td></td>
<td>Core</td>
<td>Allowed</td>
<td>General</td>
</tr>
<tr>
<td>Vc1.1-VFT</td>
<td>86.4</td>
<td>11.5</td>
<td>1.8</td>
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<tr>
<td>PeIA-VFT</td>
<td>88.4</td>
<td>10.3</td>
<td>1.1</td>
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<tr>
<td>Rg1A-VFT</td>
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<td>1.1</td>
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<td>Rg1A4-VFT</td>
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<td>7.8</td>
<td>0.1</td>
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<tr>
<td>ImI-VFT</td>
<td>92.3</td>
<td>7.4</td>
<td>0.1</td>
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</table>

$^a$ Percentage of residues belonging to the favoured (core), additionally allowed (allowed), generously allowed (general), and disallowed regions of the Ramachandran plot.

$^b$ Percentage of chi1-chi2 sidechain torsion angles, for all residue types large enough to have both these angles, which lie in favourable regions of torsion angle space.

$^c$ Percentage of residues which have average 3D-1D score $\geq$ 0.2.
Fig. S1. Docking-predicted binding location for α-conotoxin ImI at the GABA\textsubscript{B} receptor. Computational docking predicted binding of ImI, shown as large spheres at the inter-subunit region of the VFT (blue = B1, red = B2). Residues previously predicted to form close contacts with Vc1.1, RglA, and PeIA at the B1 subunit and whose Ala mutants result in significantly reduced inhibition of baclofen-sensitive current are shown as blue spheres. ImI is inactive at GABA\textsubscript{B}R, and none of the predicted binding positions allow ImI to form close direct contacts with the main canonical agonist/antagonist site residues at B1.
**Fig. S2.** Representative fluorescent micrographs of double-immunostaining of GABA<sub>B</sub> receptor residues predicted to form contact with analgesic α-conotoxins. eGFP tagged WT and other mutants of GABA<sub>B</sub>R when overexpressed recombinantly in HEK293T cells. Both GABA<sub>B1</sub> (cyan) and GABA<sub>B2</sub> (red) channel express and co-localise (merged) with each other when visualised in confocal microscopy. Right panel: DIC light microscope images of corresponding HEK293T cells. Scale bars 100 µm.
Fig. S3. Control immunocytochemistry for GABA_B1 and GABA_B2 subunits expression in HEK293T cells co-expressing Cav2.2 and eGFP tagged GABA_B along with non-transfected HEK293T cells. No immunofluorescence was detected in transfected HEK293T cells when primary antibodies directed to GABA_B1 and GABA_B2 were omitted (upper panel). Furthermore, no immunofluorescence could be detected in non-transfected HEK293T cells stained against GABA_B1 and GABA_B2 (lower panel). Lower left panels are DIC light microscope images of corresponding HEK293T cells. In all merged images, DAPI (blue) staining marks nucleus. Scale bars: 100 µm.
Fig. S4. 2-Dimensional ligand-receptor interaction diagrams. (A) α-Conotoxin RgIA (standard molecular representation) bound to GABABR displaying residues within 5Å of the peptide. Receptor residues are labelled with 3-letter codes and position numbers, and colour coded according to physicochemical properties (red = acidic, purple = basic, blue = polar, green = non-polar). Coloured lines indicate hydrogen bonding or salt-bridge interactions. Residues selected for experimental mutagenesis studies are those forming hydrogen bonds with RgIA and/or within close vicinity of such residues, and are boxed with blue (B1) or red (B2) outlines. (B) α-Conotoxin PeIA bound to GABABR. Residues selected for experimental mutagenesis studies are those forming hydrogen bonds with PeIA and/or within close vicinity of such residues, and are boxed with blue (B1) or red (B2) outlines.
**Fig. S5.** Whole-cell $I_{Ba}$ inhibition of wild-type GABA$\_R$-coupled Cav2.2 channels by baclofen, Vc1.1, Rg1A, and PeIA relative to control. Bar graphs showing whole-cell $I_{Ba}$ density (pA/pF) in the absence (control, black) and presence of 50 $\mu$M baclofen (red), and 1 $\mu$M Vc1.1 (A, blue), Rg1A (B, green), or PeIA (C, purple). Data represent mean ± SEM (n = 11 cells for each peptide). Current densities are analysed using one-way ANOVA followed by Dunnett as post hoc test where ****p < 0.0001, ***p < 0.001 and *p < 0.05.
**Fig. S6.** Lack of $\alpha$-conotoxin-induced B2 conformation shifts predicted by MD. Time series plots of B2 inter-lobe distances for GABA$_B$R bound with Vc1.1 (blue line), RglA (green), PelA (purple), and apo VFT (light grey) measured by Q292 and S352 separation. The inter-lobe separation for apo-GABA$_B$R is shown by a horizontal black line, while the separation for the baclofen-bound VFT crystal structure (4MS4) is shown with a dashed black line.
**Fig. S7.** Conformation fluctuations for apo-VFT under MD simulation. (A) Time series plot of the B1 inter-lobe separation distance measured by the proxy distance between I286 and E343. (B) Time series plot of the inter-subunit separation at the juxtamembrane region measured by the distance between B1-R239 and B2-E230.
Supplemental PDB Files

PDB 2 (RgIA4): Docking predicted binding mode of RgIA4.1 at the VFT of GABA_B receptor
PDB 2 (Vc1.1): Docking predicted binding mode of Vc1.1 at the VFT of GABA_B receptor
PDB 1 (Apo): PDB file of the apo VFT of GABA_B receptor
PDB 2 (ImI - model 1): Docking predicted binding mode of ImI at the VFT of GABA_B receptor, highest binding energy mode
PDB 3 (ImI - model 2): Docking predicted binding mode of ImI at the VFT of GABA_B receptor, second highest binding energy mode
PDB 11 (ImI - model 3): Docking predicted binding mode of ImI at the VFT of GABA_B receptor, third highest binding energy mode
PDB 10 (ImI - model 4): Docking predicted binding mode of ImI at the VFT of GABA_B receptor, fourth highest binding energy mode
PDB 9 (ImI - model 5): Docking predicted binding mode of ImI at the VFT of GABA_B receptor, fifth highest binding energy mode
PDB 8 (ImI - model 6): Docking predicted binding mode of ImI at the VFT of GABA_B receptor, sixth highest binding energy mode
PDB 7 (ImI - model 7): Docking predicted binding mode of ImI at the VFT of GABA_B receptor, seventh highest binding energy mode
PDB 6 (ImI - model 8): Docking predicted binding mode of ImI at the VFT of GABA_B receptor, eighth highest binding energy mode
PDB 5 (PeIA): Docking predicted binding mode of PeIA at the VFT of GABA_B receptor
PDB 4 (RgIA): Docking predicted binding mode of RgIA at the VFT of GABA_B receptor