Structural basis for KCTD-mediated rapid desensitization of GABAB signalling

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The GABAB (γ-aminobutyric acid type B) receptor is one of the principal inhibitory neurotransmitter receptors in the brain, and it signals through heterotrimeric G proteins to activate a variety of effectors, including G-protein-coupled inwardly rectifying potassium channels (GIRKs)1,2. GABAB-receptor signalling is tightly regulated by auxiliary subunits called KCTDs, which control the kinetics of GIRK activation and desensitization3–5. However, the mechanistic basis for KCTD modulation of GABAB signalling remains incompletely understood. Here, using a combination of X-ray crystallography, electron microscopy, and functional and biochemical experiments, we reveal the molecular details of KCTD binding to both GABAB receptors and G-protein βγ subunits. KCTDs associate with the receptor by forming an asymmetric pentameric ring around a region of the receptor carboxy-terminal tail, while a second KCTD domain, H1, engages in a symmetric interaction with five copies of Gβγ in which the G-protein subunits also interact directly with one another. We further show that KCTD binding to Gβγ is highly cooperative, defining a model in which KCTD proteins cooperatively strip G proteins from GIRK channels to induce rapid desensitization following receptor activation. These results provide a framework for understanding the molecular basis for the precise temporal control of GABAB signalling by KCTD proteins.

The GABAB receptor (GABABR) is expressed in excitatory and inhibitory synapses throughout the brain, and it is an important target for anxiolytic and antispasitic drugs1,2. The receptor is a heterodimer composed of GABAB1 and GABAB2 subunits, both of which are essential for the formation and trafficking of a functional receptor4. Upon activation, the receptor catalyses the dissociation of heterotrimeric G proteins into Gα, Gβγ, and Gγ subunits to initiate downstream signalling5–8. Following activation of presynaptic GABABRs, the Gγ heterotrimer typically inhibits voltage-gated calcium channels (VGCCs) to suppress neurotransmitter release, while activation of postsynaptic GABABRs usually activates outward potassium conductance via GIRK9–11 to generate slow inhibitory postsynaptic currents (sIPSCs)11,12.

GABAB signalling is controlled by the proteins KCTD8, KCTD12 and KCTD16, which serve as auxiliary receptor subunits that regulate the rise time and duration of GABAB receptor and enhance receptor expression levels1–5. These effects are mediated by KCTD interaction with the C terminus of the GABABγ subunit and with Gβγ proteins4–13. KCTDs are genetically associated with mood disorders including bipolar disorder14, and their heterogeneous subtype expression patterns serve to create functionally distinct populations of GABAB receptors in different brain regions15. Despite their importance, the mechanisms by which KCTDs bind to the GABABγ subunit and sequester G proteins are ill-defined. Here we sought to tackle these problems to define the structural and mechanistic basis for KCTD function.

KCTD8, KCTD12 and KCTD16 consist of an amino-terminal BTB domain (a protein–protein interaction motif), followed by a region of unknown structure termed the H1 domain. The BTB domain mediates binding to GABAB receptors at the distal receptor tail, centred around GABAB2 residue Tyr903 (we use human sequence numbering throughout; this residue is equivalent to Tyr902 in the mouse)13,16. We sought to identify a GABAB2 peptide fragment that is capable of binding to a KCTD protein. Using a pull-down assay, we found that a peptide consisting of GABAB2 residues 876–913 could bind to KCTD16BTB (Fig. 1a and Extended Data Fig. 1a, b), and we determined the crystal structure of this peptide in complex with KCTD16BTB to 3.2 Å resolution (Fig. 1b, Extended Data Table 1 and Extended Data Fig. 2).

The structure shows an unusual overall architecture, with KCTD16BTB adopting an asymmetric pentameric arrangement and wrapping around the GABAB2 C terminus (Fig. 1b). Although previous reports had suggested that KCTD16 functions as a tetramer, both our structure and a recently reported peptide-free KCTD16BTB structure17 show a pentameric arrangement (Extended Data Fig. 1c). Over a stretch of roughly 25 amino acids, nearly every side chain in the GABABγ tail interacts with KCTD16BTB (Fig. 1b). Within the interface, KCTD16BTB residue Phe80 makes particularly extensive contacts with the peptide in each of the five subunits (Fig. 1c). Confirming the importance of these interactions, we found that an F80A mutation in KCTD16BTB caused a complete loss of GABAB2 peptide binding activity in vitro (Extended Data Fig. 1d), and an equivalent substitution in KCTD12 resulted in loss of GABAB- mediated KCTD12 membrane localization in cells (Fig. 1d). Almost all residues in the interaction interface are conserved in all GABAB-regulating KCTD subtypes (KCTD8, KCTD12 and KCTD16), accounting for the specific binding of these KCTDs to GABAB receptors (Extended Data Fig. 1e).

In addition to binding the GABABγ tail, KCTDs also interact with Gγ subunits4. In an in vitro pull-down assay using glutathione-S-transferase (GST)-tagged KCTD12, we found that a substoichiometric amount of GABAB2 receptor tail but near-stoichiometric amounts of Gγ1,2 heterodimer could be pulled down by KCTD12. By contrast, KCTD12 failed to co-purify Go1,2 heterotrimers irrespective of Go subtype (Fig. 2a and Extended Data Fig. 3a, b). While the KCTD12 BTB domain mediates interaction with the receptor, its C-terminal H1 domain is responsible for desensitization18. Consistent with this, we observed that the KCTD12 H1 domain alone was sufficient to bind Gγ1,2 with an affinity of 185 nM (Fig. 2b and Extended Data Fig. 3c).

Negative-stain electron-microscopy two-dimensional class averages of the KCTD12H1 complex in complex with Gγ1,2 revealed a symmetric pentameric assembly, with the H1-domain pentamer engaging in interaction with five copies of the Gγ1,2 heterodimer (Fig. 2b and Extended Data Fig. 3d–f).

To investigate the details of H1-domain interaction with Gγ1,2 in more detail, we determined the crystal structure of the KCTD12H1/Gγ1,2 complex to 3.7 Å resolution (Extended Data Table 1 and Extended Data Fig. 2b). In parallel, we determined a negative-stain electron-microscopy envelope for full-length KCTD12 in complex with Gγ1,2 (Extended Data Fig. 4). These data show that the KCTD12H1/Gγ1,2 complex is pentameric and displays near-perfect C5 symmetry. KCTD12H1 possesses a β-propeller-like fold, with each H1 domain comprising a separate blade of the propeller. The propeller is surrounded by a tightly

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7 MARCH 2019 | VOL 567 | NATURE | 127
Fig. 1 | Crystal structure of the KCTD16 BTB domain in complex with a peptide from the GABA_{B2} C terminus. a, Left, schematic representation of the domain organization of the GABA_{B2} receptor, KCTD12 and KCTD16. 7TM, seven transmembrane domain. Right, summary of GST pull-down results using GST-tagged GABA_{B2} fragments and the KCTD16 BTB domain. b, Ribbon representation of the structure of the KCTD16_{BTB}/GABA_{B2} peptide complex. c, Detailed view of the interface indicated by the black box in b. d, Subcellular localization of wild-type (WT) GFP–KCTD12 or mutant GFP–KCTD12(F87A) (equivalent to F80A in KCTD16) with or without the GABA_{B} receptor in HEK293T cells. Fluorescence intensity was measured across the red line through a representative cell and is plotted below. AU, arbitrary units.

Fig. 2 | Crystal structure of the KCTD12 H1 domain and G_{3}γ_{2} complex. a, GST pull-down experiment. The input (IN) and pull-down (PD) fractions were separated by SDS–PAGE and stained with Coomassie blue. Cter, C-terminal peptide of GABA_{B2} (residues 876–913). b, Left, size-exclusion chromatography analysis of individual domains and complexes shows the assembly of large heteromeric complexes.
Mechanism of recognition of G\(\beta\gamma\) by the KCTD12 H1 domain.

**Fig. 3**

- a. The two KCTD12\(\alpha\) subunits contact distinct surfaces of G\(\beta\gamma\), denoted interface I and interface II.
- b. Interactions in interface I.
- c. Interactions in interface II.
- d. KCTD12\(\alpha\) R232D or R257D mutations abolish the interaction with G\(\beta\gamma\) in size-exclusion chromatography.
- e. Whole-cell patch-clamp GIRK current traces recorded from Chinese hamster ovary (CHO) cells co-expressing the GABA\(\beta\) receptor, GIRK1 and wild-type or mutant GFP-labelled KCTD12. 
- f. Relative desensitization levels, shown as means ± s.e.m. Each data point is from one cell; for full statistical details see Methods.
- g. KCTD12-, GRK2-, GIRK- and G\(\beta\) binding interfaces circled on G\(3\gamma\).
- h. The interface with the GIRK channel is distinct from, and does not overlap with, the KCTD12 H1 interface on G\(\beta\).

packed ring of G\(\beta\gamma\) heterodimers in direct contact with one another. Interactions between KCTD12 and the G protein are confined to the G\(\beta\) subunit, with G\(\gamma\) subunits arrayed peripherally around the edge of the pentameric ring. Previous reports had suggested that the amino-acid motif NFLEQ in KCTD12 is important for receptor desensitization\(^{18}\). However, in our crystal structure this region does not interact with G\(3\gamma\) subunits, but is instead involved in oligomeric contacts between adjacent KCTD12 subunits (Extended Data Fig. 5a).

The KCTD12 H1 domain itself consists of a five-stranded antiparallel \(\beta\)-sheet interrupted by two \(\alpha\)-helices (Extended Data Fig. 5b). The five G\(3\gamma\) subunits each interact with two KCTD12\(\alpha\) subunits, which together occlude a large surface on G\(3\) (Fig. 3a and Extended Data Fig. 5c). The loop between the first and second \(\beta\)-strands of KCTD12\(\alpha\) includes basic residues that interact with an acidic patch on the G\(3\) \(\beta\)-propeller (Fig. 3b); a longer loop in KCTD12\(\alpha\) after the first \(\alpha\)-helix sits in the groove between the N-terminal helix of G\(3\) and the \(\beta\)-propeller domain (Fig. 3c). Mutating residues in the KCTD12 H1 domain in either of these interfaces (R232D or R257D mutations) entirely abolishes G\(\beta\) protein binding (Fig. 3d). Although both KCTD2 mutants can localize to the cell membrane in the presence of GABA\(\beta\) receptors (Extended Data Fig. 5d), they fail to desensitize GABA\(\beta\)-mediated GIRK channel currents (Fig. 3e, f). Interestingly, the GIRK-binding site on the surface of G\(3\gamma\) would partially occlude G\(\alpha\) binding (Fig. 3g), but is entirely non-overlapping with the GIRK-channel-binding site\(^{19}\) (Fig. 3g, h).

Hence, binding of a KCTD12 pentamer to a single G\(3\gamma\) subunit in complex with GIRK2 could be accommodated with minimal structural rearrangement, although the full 5/5 complex is incompatible owing to extensive clashes between the GIRK2 intracellular domain and other KCTD12-bound G\(3\gamma\) dimers (Fig. 3h and Extended Data Fig. 6a, b).

One of the more remarkable features of the complex is the fact that the G\(3\gamma\) subunits pack so closely to one another that they interact directly, burying a surface of 351 Å\(^2\). By contrast, the GIRK channel is bound by four spatially isolated G\(3\gamma\) subunits (Extended Data Fig. 6a–c). Residues in the G\(3\)–G\(3\) interface observed in our structure are highly conserved across species, raising the possibility that this interaction is functionally important. To test this, we incubated KCTD12\(\alpha\) with a substoichiometric amount of G\(3\gamma\). Under these conditions, only full 5/5 complexes and free KCTD12 were seen with size-exclusion chromatography, with no evidence of partial oligomers, suggesting that KCTD12 binding to G\(3\gamma\) is highly cooperative (Extended Data Fig. 6d). When a single G\(3\) residue involved in oligomeric G\(3\)–G\(3\) contacts was mutated (R42D mutation), we observed a broad 5/5 complex peak and free G\(3\gamma\) in size-exclusion chromatography. Introduction of a second mutation (R46D) resulted in only partial complex formation (Extended Data Fig. 6e), further supporting a role of cooperative G\(3\)–G\(3\) contacts in KCTD binding.

G\(3\gamma\) is the key signalling intermediate during GABA\(\beta\) activation of GIRK currents, and in the course of a signalling event a G\(3\gamma\) subunit interacts sequentially with a series of progressively higher-affinity binding partners. The dissociation constants (\(K_d\)) for G\(3\gamma\) binding
to GTP-bound Goα, GIRK channels, KCTD12 and GDP-bound Goi are (respectively): undetectable, roughly 250 μM (ref. 20,21), 185 nM (Extended Data Fig. 3c), and 3 nM (ref. 22). If the affinity of G or Gα for KCTDs is so much higher than its affinity for GIRK channels, how can KCTDs desensitize the channels without preventing their activation in the first place? Clearly, the on-rate for Gα or Gαi association with KCTDs must be lower than that for association with GIRK channels, otherwise Gα or Gαi subunits would be sequestered prematurely. We propose one possible explanation for how this might be achieved.

KCTDs bind at the distal C terminus of the GABA-receptor B2 subunit—roughly 350 Å from the last transmembrane domain—with an intervening, structurally rigid coiled-coil domain. This means that KCTDs can diffuse far from the membrane plane (Fig. 4). When the GABA-receptor is activated, Gα or Gαi subunits are liberated from Go but remain tethered to the membrane because of their lipid anchor, resulting in GIRK channel activation while KCTDs are at a relatively low local concentration. When a KCTD molecule happens to encounter a GIRK-bound Gα or Gαi subunit, it can bind simultaneously, but formation of the oligomeric complex is sterically incompatible with Gα or Gαi binding to GIRK channels. The higher affinity and cooperativity of binding to KCTD may then drive stripping of subsequent Gα or Gαi subunits from GIRKs following an initial encounter, resulting in rapid channel closure. Finally, reassociation of Gα or Gαi with GDP-bound Go subunits could restore equilibrium and reset the cycle (Extended Data Fig. 7).

The regulation of GABARs signalling by KCTD proteins is a unique and important means of achieving functional diversity in these critical receptors. Here we show how the KCTD BTB domain recognizes the GABAR C terminus to tether a Gαi-sequestering H1 domain on the receptor’s distal tail, providing a means of efficiently deactivating GIRK channels. In this way, KCTDs afford GABAR receptors with tight temporal control of signalling that is not achievable with the slower β-arrestin pathway used to desensitize most GPCRs.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41586-019-0990-0.

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METHODS

Data reporting. No statistical methods were used to predetermine sample size. The experiments were not randomized and the investigators were not blinded to allocation during experiments and outcome assessment.

Protein expression and purification. Human KCTD12 and KCTD16 genes were synthesized by Integrated DNA Technologies. KCTD12 (residues 32–131) and KCTD16 (residues 23–124) BTB domain were cloned into a pET28a expression vector (EMD Millipore) to allow expression of a fusion protein consisting of the KCTD BTB domain plus an N-terminal His6–SUMO tag, or expression of an untagged BTB domain, in *Escherichia coli* strain BL21(DE3). KCTD12 (residues 200–325) and KCTD16 (residues 158–279) H1 domains were cloned into the multiple cloning site 1 of pETduet-1 vector (EMD Millipore) and expressed with an N-terminal His6–SUMO tag followed by a 3C protease site. All human GABAB2 C-terminal truncations were cloned into the same vector with an N-terminal His6–GST or His6–SUMO fusion protein followed by a 3C protease site, and were expressed in BL21(DE3) *E. coli*. KCTD12 (residues 32–325) and KCTD16 (residues 21–279) constructs containing both BTB and H1 domains were cloned into the pVLI39 vector (Expression Systems) and protein was expressed using baculovirus infection of Sf9 insect cells (Expression Systems) according the manufacturer's protocols. These constructs were expressed with an N-terminal His6–GST fusion protein followed by a 3C protease site. Human GCD2 was cloned into pVLI39 without tags. Human Gα16/γ2 heterodimer was modified to include an N-terminal His6 tag on the G7 subunit and a G68S point mutation in G7, which eliminates the lipid-modification site. A bicistronic vector based on pVLI39 was used to prepare baculovirus encoding both subunits. All point mutations and deletions were introduced by QuikChange Lightning method. Data were processed in the HKL2000 software package25. Crystals of KCTD16p22 in complex with the GABAB2 fragment belonged to the P21 space group, and contained two copies of the KCTD16 BTB/GABAB2 fragment complex per asymmetric unit. Phase was determined with molecular replacement using Phaser in Phenix26 with the KCTD16BTB structure (Protein Data Bank [PDB] identification code 5A15) as a search model. The model refined structure includes two copies of the KCTD16BTB pentamer with residues 23–124 resolved, and two GABAB2 C-terminal peptides with residues 884–913 resolved. Ramachandran analysis showed that 95.7% of residues are in the favoured regions, and 4.3% in the allowed regions. Crystals for the KCTD12/Gβ2 complex belong to space group P212121. The structure was solved by molecular replacement using Phaser in Phenix with the structure of the bovine Gα16/γ2 heterodimer (PDB identification code 1OMW) as a search model. The model includes one copy of the KCTD12 H1 pentamer (residues 206–324) and five copies of the Gα16/γ2 complex. The model was refined through iterative model building in Coot28 and reciprocal space refinement in phenix.refine22. The final structure has 97.36% and 2.64% of residues in the favoured and allowed regions, respectively, of the Ramachandran plot.

Isothermal titration calorimetry. Isothermal titration calorimetry (ITC) experiments were performed with an ITC200 microcalorimeter (Microcal) at 30 °C. Purified KCTD12α1 and Gβ2 were exchanged to the same buffer (20 mM HEPES-NaOH pH 7.6 and 100 mM NaCl) with a Hitrap Desalting column (GE Healthcare) before titration. We injected 160 μM of Gβ2 via syringe into a sample cell containing 16 μM of KCTD12α1. The integrated heat data were analysed using the one-set-of-sites model in Origin according to the manufacturer's instructions. The dissociation constant Kd was calculated as 1/association constant (1/Ka).

Electron microscopy and three-dimensional reconstruction. For negative staining, 2.5 μl of protein solution consisting of a complex of GABAB2 C-terminal peptide, full-length KCTD12 and Gβ2 at a concentration of about 0.01 mg ml−1 was added to a glow-discharged carbon-coated copper grid (Electron Microscopy Science) and allowed to adsorb for 20 s. Grids were then washed twice with deionized water and stained twice with freshly prepared 0.75% (w/v) uranyl formate. Filter paper was applied to absorb residual liquid between each step. Images were collected at room temperature using a Philips Tecnai T12 electron microscope equipped with an LaB6 filament and operated at 120 kV. Images were collected at a magnification of 20,000-fold and a defocus value of 1.5 μm. A Gaian 4K charge-coupled-device (CCD) camera using a low-dose collection procedure. Particles were manually picked in Eman2 (ref. 39) and two-dimensional class averages were calculated with Relion31. Three-dimensional initial model building, three-dimensional classification and refinement were carried out in cisTREM2.

Imaging of HEK293T cells. Wild-type or mutant KCTD12 was cloned into pCDNA3.1 vector for the expression of an N-terminal protein C (PC–KCTD12) or enhanced green fluorescent protein (eGFP–KCTD12) fusion protein. HEK293T cells were cultured in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and maintained at 37 °C in a 5% CO2 humidified incubator. Cells were seeded on poly-L-lysine coated 18-mm coverslips 18 h before transfection. Transfections were done using lipofectamine 3000 (Invitrogen) with 0.7 μg per well of eGFP–KCTD12, eGFP–KCTD12(F87A),
The number of cells measured per condition is as follows: minus KCTD, 13; plus per condition is as follows: minus KCTD, four; plus KCTD, two; plus eGFP–KCTD, Devices) and Prism (GraphPad). The number of experimental replicates Bessel (8-pole) at 2 kHz. Recordings were analysed using Clampfit (Molecular HEPES, 10 mM Tris phosphocreatine, 5 mM EGTA, 4 mM Na₂ATP and 0.6 mM sodium bicarbonate and 11 mM D-glucose (pH 7.2), and maintained at 37 °C in a 5% CO₂ humidified incubator. Cells were seeded on poly-L-ornithine-coated 18-mm coverslips and transfected roughly 24 h later. Transfections were done using lipofectamine 2000 (Invitrogen) with 0.7 μg of DNA encoding KCTD12, eGFP–KCTD12, eGFP–KCTD12(F87A), eGFP–KCTD12(R232D) or eGFP–KCTD12(R257D), 0.7 μg GIRK1(F137S) (homotetramerization mutant), 0.3 μg tdTomato fluorescent protein, and 0.35 μg KCTD12(R257D), 0.7 μg g GIRK1(F137S) (homotetramerization mutant), 0.3 μg g each of rat GABAB1A and GABA B2. Cells were maintained in 2 μM CTP 54626 hydrochloride post-transfection to reduce basal activity of GABA B and to maintain cell health.

Whole-cell patch-clamp electrophysiology. CHO cells were cultured in nutrient mixture F-12 Ham (Sigma–Aldrich) supplemented with 10% FBS, 14 mM sodium bicarbonate and 11 mM D-glucose (pH 7.2), and maintained at 37 °C in a 5% CO₂ humidified incubator. Cells were seeded on poly-L-ornithine-coated 18-mm coverslips and transfected roughly 24 h later. Transfections were done using lipofectamine 2000 (Invitrogen) with 0.7 μg of DNA encoding KCTD12, eGFP–KCTD12, eGFP–KCTD12(F87A), eGFP–KCTD12(R232D) or eGFP–KCTD12(R257D), 0.7 μg GIRK1(F137S) (homotetramerization mutant), 0.3 μg tdTomato fluorescent protein, and 0.35 μg each of rat GABAB1A and GABA B2. Cells were maintained in 2 μM CTP 54626 hydrochloride post-transfection to reduce basal activity of GABA B and to maintain cell health.

Whole-cell patch-clamp experiments were performed 42–72 h after transfection in an extracellular solution containing 120 mM KCl, 25 mM NaCl, 10 mM HEPES, 2 mM CaCl₂ and 1 mM MgCl₂ (pH 7.4). GABA B responses were induced with a 488-nm laser diode. Images were saved as 16-bit TIFF files. A region of interest (ROI) was drawn around a blank area lacking cells to perform background subtraction, using a macro on Fiji (ImageJ) that subtracts the mean intensity value of the ROI from the image. Line-scan analysis was performed on background-subtracted images.

Data availability

The refined coordinates and structure factors for the KCTD16BTB/GABA B2 peptide complex and KCTD12B/G/B2 complex have been deposited in the Protein Data Bank under the accession codes 6M8R and 6M8S, respectively.

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Extended Data Fig. 1  | Mapping the GABA_{B2}-binding region of the KCTD BTB domain. 

**a**, KCTD16_{BTB} or KCTD12_{BTB} domains tagged with His_{6}–SMT3 (a SUMO protein) and GABA_{B2} fragments tagged with His_{6}–GST were coexpressed in *E. coli* and purified with nickel affinity chromatography. Purified protein was loaded as input for pull-down with glutathione–sepharose beads. Glutathione input (IN) and pull-down (PD) fractions were analysed by SDS–PAGE and Coomassie blue staining.

**b**, His_{6}–GST-tagged GABA_{B2} fragments and untagged KCTD16 BTB were coexpressed in *E. coli*. Clarified lysate was pulled down with glutathione–sepharose beads and eluate was analysed by SDS–PAGE and Coomassie blue staining.

**c**, Structural superposition of the KCTD16_{BTB} structure as bound to GABA_{B2} peptide (red; PDB identification 5A15) or free of peptide (grey).

**d**, His_{6}–SMT3-tagged wild-type or mutant (F80A) KCTD16_{BTB} and His_{6}–GST-tagged GABA_{B2} peptides were coexpressed and purified with nickel affinity. The eluate was treated with the protease ULP1 to cleave the SMT3 tag (IN) before pull-down (PD) using glutathione–sepharose beads.

**e**, Sequence alignment of the BTB domains from human KCTD-family members. Residues with 98%, 80% and 60% similarity are shown in black, grey and light grey, respectively.
Extended Data Fig. 2 | Electron-density maps. a, Composite omit $2F_o - F_c$ electron-density map, contoured at 1.0σ, for a GABA$_{B_2}$ C-terminal peptide in complex with the KCTD16$_{BTB}$ domain. b, $2F_o - F_c$ electron-density map, contoured at 1.0σ, for the KCTD12$_{SH}$ domain in complex with G$\delta_1$$\gamma_2$. 
Extended Data Fig. 3 | Representative negative-stain electron micrographs. a, The full SDS–PAGE gel for the pull-down experiment shown in Fig. 2a. b, Goi competes with KCTD12 for binding of Gβγ2. These pull-down experiments were carried out as for Fig. 2a, except that Goi was used instead of Gαq. c, Isothermal titration calorimetry affinity measurements for the binding of KCTD12H1 to Gβγ2. d, Representative negative-stain electron-microscopy two-dimensional image of KCTD16BTB+H1 bound to the C-terminal GABA32 domain. e, Representative negative-stain electron-microscopy two-dimensional image of KCTD12H1/Gβγ2. f, Representative negative-stain electron-microscopy two-dimensional image of KCTD12BTB+H1/GABA32Cter/Gβγ2.
Extended Data Fig. 4 | Three-dimensional negative-stain electron-microscopy reconstruction of full-length KCTD12 in complex with the GABAβ2 C-terminal domain and Gβ1γ2. a, Bottom, three different views of a three-dimensional reconstruction of full-length KCTD12 in complex with Gβ1γ2, with crystal structures of KCTD16BTB/GABAβ2 peptide (red) and KCTD12H1/Gβ1γ2 (blue/purple) docked into the negative-stain electron-microscopy envelope. Top, negative-stain electron-microscopy two-dimensional class averages are reproduced from Fig. 2b for reference. b, Fourier shell correlation (FSC) curve as a function of spatial frequency for a negative-stain electron-microscopy map. Resolution is indicated below. c, Structure of the full-length KCTD12 complex. The BTB domain is separated from the H1 domain by 35 Å. Dashed lines represent the linker sequence between the BTB and H1 domains.
Extended Data Fig. 5 | Structural and functional analysis of H1 domain.

**a.** Ribbon representation of the KCTD12_H1 domain. The NFLEQ motif, which is important for desensitization, is coloured orange.

**b.** Ribbon representation and topology map of the KCTD12 H1 monomer. Elements of the secondary structure are labelled.

**c.** Ribbon representation of two KCTD12_H1 subunits (green and cyan) and Gβ_1γ_2.

**d.** Subcellular localization of eGFP–KCTD12(R232D) and eGFP–KCTD12(R257D) mutants with or without GABA_A receptor.
Extended Data Fig. 6 | Binding of G\(\beta\gamma\) to KCTD12 is highly cooperative. a, Ribbon representation of a complex of GIRK and G\(\beta\gamma\) as seen from the cytosolic side. Four isolated G\(\beta\gamma\) heterodimers associate with a single GIRK tetramer. b, Ribbon representation of a complex of KCTD12\(\text{H1}\) and G\(\beta\gamma\) as viewed from the cytosolic side. G\(\beta\gamma\) subunits can be seen to interact directly with one another. c, Interactions between two adjacent G\(\beta\) subunits (labelled 1 and 2). d, 0.5/5, 1/5 or 5/5 molar ratios of G\(\beta_1\gamma_2\) to KCTD12\(\text{H1}\) were mixed and subjected to size-exclusion separation. The peaks correspond to 5/5 complexes and H1 domain alone. The x axis shows the elution volume (in ml). e, Left, a stoichiometric amount of G\(\beta_1\gamma_2\) carrying a single mutation, R42D (in the G\(\beta_1\) subunit), or a double mutation, R42D/R46D, was incubated with KCTD12\(\text{H1}\) and analysed by size-exclusion chromatography. Right, fractions from peak 1, peak 2 and peak 3 were analysed by SDS–PAGE and correspond to a 5/5 full complex, a partial complex and free G\(\beta_1\gamma_2\), respectively.
Extended Data Fig. 7 | A model for GABA<sub>B</sub> signalling and desensitization. a, From left, binding of agonist (red star) to the GABA<sub>B1</sub> receptor causes the $G_{\beta\gamma}$ heterodimer to dissociate from $G_{\alpha}$. Four copies of $G_{\beta\gamma}$ bind to a GIRK channel tetramer, resulting in channel activation and an outflow of $K^+$. Afterwards, KCTD bound to the GABA<sub>B2</sub> C terminus strips four copies of $G_{\beta\gamma}$ from the GIRK channel and thereby deactivates the channel. Following nucleotide (GTP) hydrolysis by adenyl cyclase, GDP-bound $G_{\alpha}$ binds again to $G_{\beta\gamma}$, sequestering it from KCTD and priming the system for another signalling cycle. b, Calculated total energy change for the series of progressively tighter binding events is commensurate with the approximate energy released by GTP hydrolysis.
Extended Data Table 1 | Crystallographic statistics

| Data collection | KCTD16_BTG/GABAter | KCTD12_H/ Gβγ2 |
|-----------------|---------------------|-----------------|
| Space group     | $P2_1$              | $P2_12_12_1$    |
| Cell dimensions |                     |                 |
| $a$, $b$, $c$ (Å) | 92.5, 65.1, 114.1  | 109.1, 122.0, 206.4 |
| $\alpha$, $\beta$, $\gamma$ (°) | 90.0, 99.6, 90.0 | 90.0, 90.0, 90.0 |
| Resolution (Å)  | 50 - 3.2 (3.28 - 3.20) | 50 - 3.71 (3.80 - 3.71) |
| $R_{merge}$ (%) | 33.2 (99.7)         | 31.2 (105.9)    |
| CC1/2 (%)       | 92.6 (56.1)         | 96.2 (52.0)     |
| $I / \sigma I$  | 3.4 (0.6)           | 3.56 (1.09)     |
| Completeness (%)| 99.2 (96.7)         | 99.0 (97.6)     |
| Redundancy      | 3.3 (2.7)           | 3.9 (4.0)       |

| Refinement      |                     |                 |
|-----------------|---------------------|-----------------|
| Resolution (Å)  | 38.6-3.2 (3.29-3.20) | 48.4-3.71 (3.76-3.71) |
| No. reflections | 22002               | 29614           |
| $R_{work}$ / $R_{free}$ (%) | 23.6 / 27.6 | 25.6 / 28.1 |
| No. atoms       | 8462                | 18990           |
| Protein         | 8462                | 18990           |
| Ligand/ion      | 0                   | 0               |
| Water           | 0                   | 0               |
| $B$-factors     | 57.3                | 93.0            |
| Protein         | 57.3                | 93.0            |
| Ligand/ion      | N/A                 | N/A             |
| Water           | N/A                 | N/A             |
| R.m.s. deviations | Bond lengths (Å) | 0.005           |
|                 | Bond angles (°)     | 1.095           |

Values in parentheses are for highest shell. N/A, not applicable; r.m.s., root mean square.
Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
- Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

| Data collection | Software used for X-ray and electron microscopy data processing is described in the methods section with references to relevant literature. All software was supported by the SBGrid consortium and no custom code was used. |
| Data analysis    | Software used for X-ray and electron microscopy data processing is described in the methods section with references to relevant literature. All software was supported by the SBGrid consortium and no custom code was used. |

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Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The refined coordinates and structure factors for the KCTD16 BTB domain complex with GABAB2 c-terminal peptide are deposited in the protein data bank under accession code 6M8R. Refined coordinates and structure factors for the KCTD12 H1 domain in complex with G beta1 gamma2 are deposited under accession core 6M8S.
Field-specific reporting

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☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | N/A |
|-------------|-----|
| Data exclusions | N/A |
| Replication | All attempts at replication have been successful. Gel images and EM micrographs are representative examples. |
| Randomization | N/A |
| Blinding | No blinding. |

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

### Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☒ Antibodies | ☐ |
| ☒ Eukaryotic cell lines | ☐ |
| ☒ Palaeontology | ☐ |
| ☒ Animals and other organisms | ☐ |
| ☒ Human research participants | ☐ |
| ☒ Clinical data | ☐ |

### Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☒ ChIP-seq | ☐ |
| ☒ Flow cytometry | ☐ |
| ☒ MRI-based neuroimaging | ☐ |

Eukaryotic cell lines

Policy information about cell lines

| Cell line source(s) | Cell lines were purchased directly from ATCC |
|---------------------|--------------------------------------------|
| Authentication      | Cell lines were not independently authenticated |
| Mycoplasma contamination | All cell lines were tested and confirmed negative for mycoplasma contamination |
| Commonly misidentified lines (See ICLAC register) | N/A |

(See ICLAC register)