Supplementary information, Figure S1

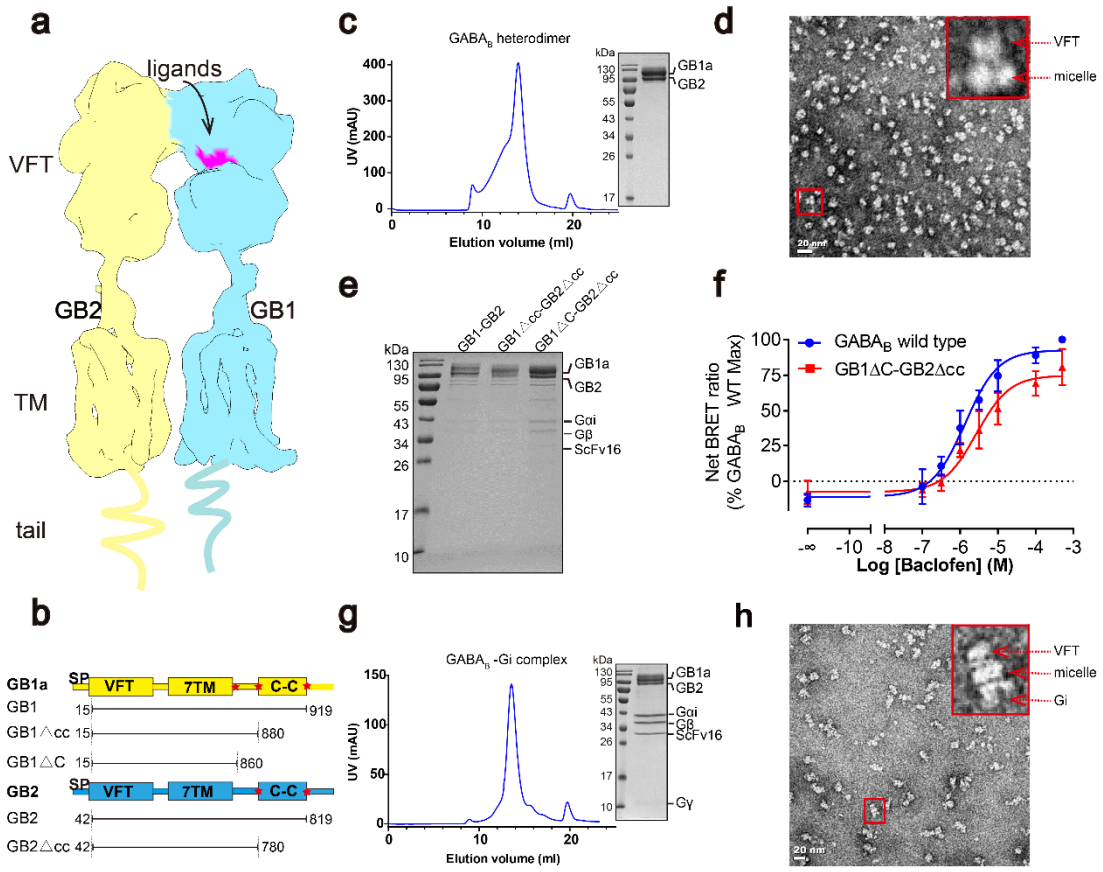

Fig. S1 Purification and characterization of the GABA$_B$ heterodimer and GABA$_B$-G$_{i1}$ complex. a Schematic diagram of the heterodimeric GABA$_B$ receptor. **b** Schematic diagram of constructs used in this study. Δcc represents the coiled-coil (c-c) domain is removed. ΔC represents the entire C-terminus after TM domain in GB1 or GB2 is truncated. **c** Size-exclusion chromatography profile and SDS-PAGE gel of the purified antagonist-bound GABA$_B$ receptor. **d** Negative staining EM analysis of the purified inactive GABA$_B$ receptor shows the clear density for VFT and detergent micelles. **e** G protein pull-down analysis of the different truncations (GB1-GB2, GB1Δcc-GB2Δcc, and GB1ΔC-GB2Δcc) of GABA$_B$ receptor. SDS-PAGE gel showed that GB1ΔC-

GB2Δcc exhibits a greater efficiency in complex formation among these constructs and were selected for further structural study. **f** Dose-response curve of baclofen-induced BRET changes in the Gαi sensors for the wide-type and GB1ΔC-GB2Δcc construct of $GABA_B$ receptor ($GABA_B$Δcc), showing that $GABA_B$Δcc exhibits similar pharmacology to that of wild-type receptor. **g** Size-exclusion chromatography profile and SDS-PAGE gel of the purified agonist/PAM bound $GABA_B$–G_{i1} complex using GB1ΔC-GB2Δcc construct. **h** Negative staining EM visualization of the purified $GABA_B$-G_{i1} complex showing clear density for VFT, detergent micelles and G_{i1} protein.