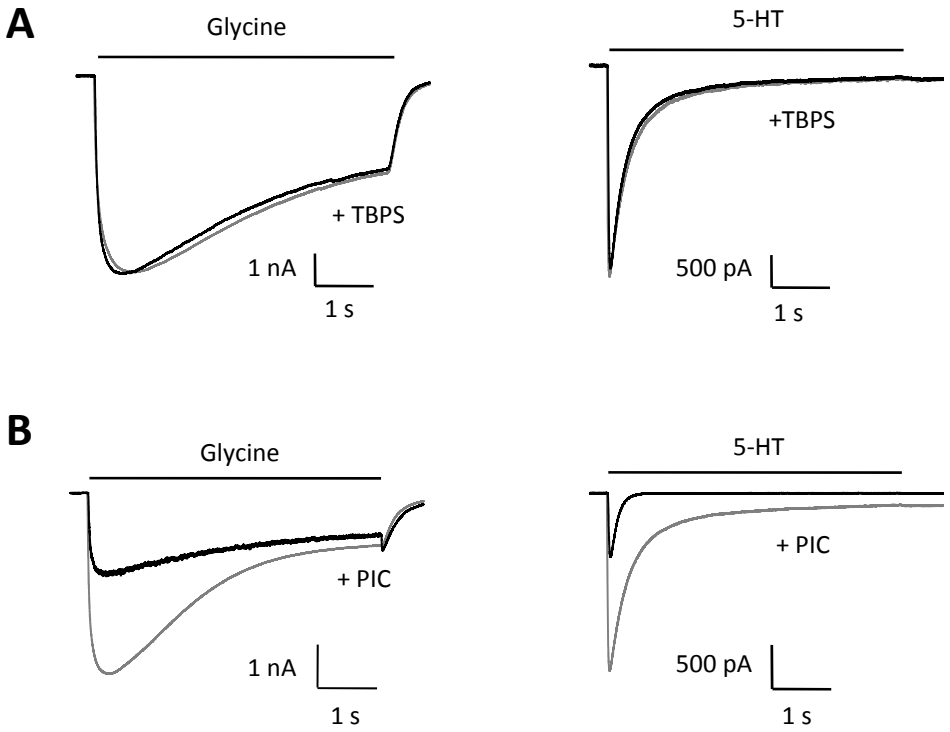
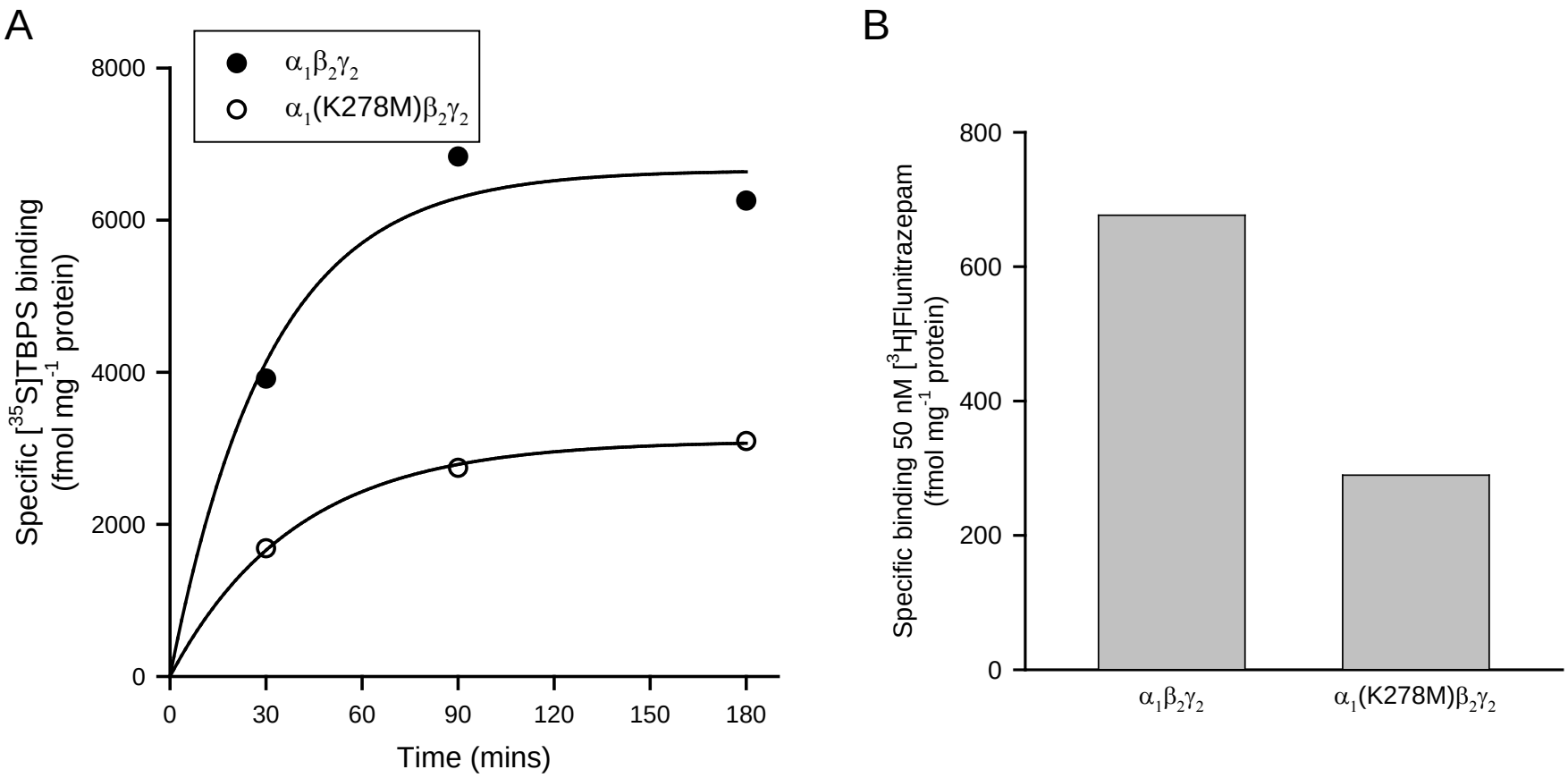
Supplementary Figure 1



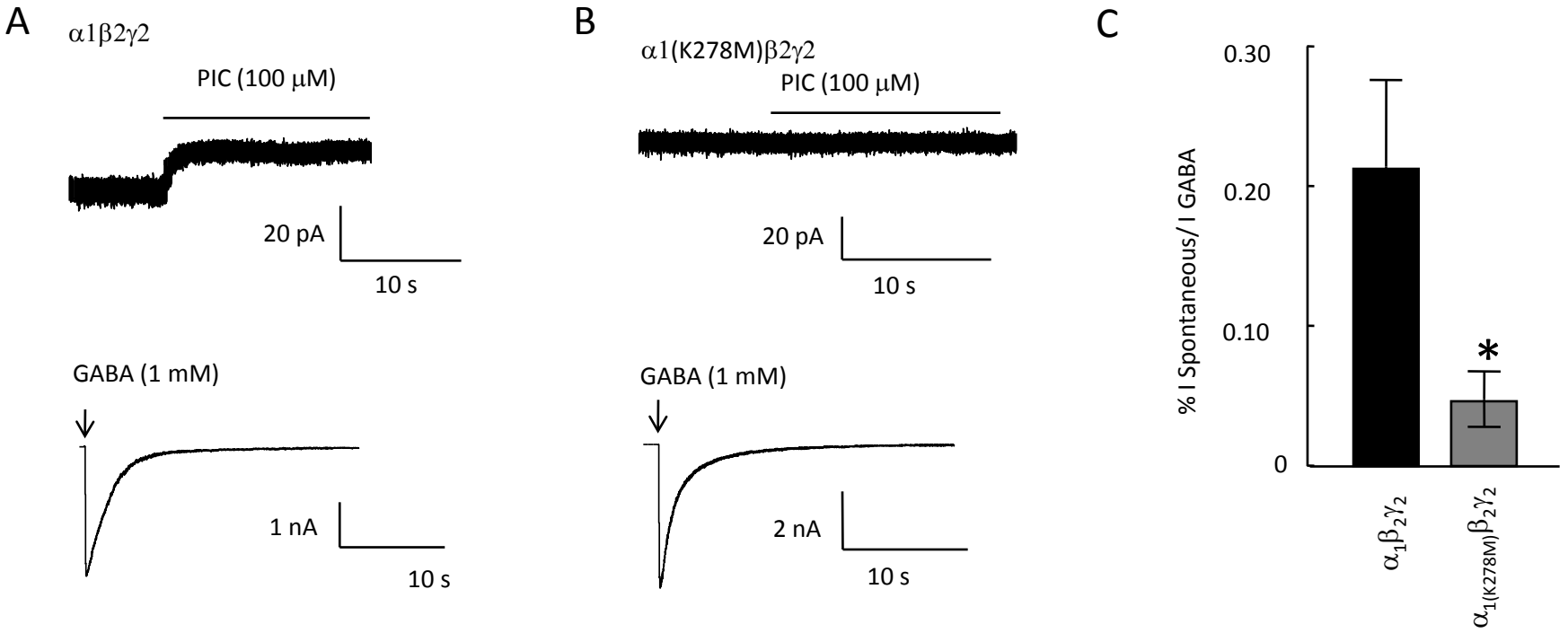
Supplementary Figure 1. The effects of TBPS and picrotoxin on 5-HT_3A and glycine α2 receptors. A. TBPS (10 mM) applied at a concentration that caused near maximal block of $GABA_A$Rs (Fig. 1) had no effect on currents mediated by either α2 glycine or 5-HT_3A receptors expressed in HEK293 cells ($n \geq 3$). B. Picrotoxin (100 μM) inhibited currents evoked by 5-HT (100 μM) and glycine (100 μM) recorded from HEK293 cells expressing 5-HT_3A or glycine α2 receptors, respectively.

Supplemental Figure 2



Properties of TBPS and benzodiazepine binding to wild-type and mutant $GABA_A$ receptors. **A**. Time course of [^{35}S]TBPS binding to $\alpha_1\beta_2\gamma_2$ or α_1(K278M)$\beta_2\gamma_2$ $GABA_A$ receptors. HEK membranes containing either $\alpha_1\beta_2\gamma_2$ or α_1(K278M)$\beta_2\gamma_2$ $GABA_A$ receptors were incubated with 30 nM [^{35}S]TBPS for 30, 90 or 180 mins. The data were fitted with an exponential function to determine the time course of obtaining equilibrium binding. The time constants for [^{35}S]TBPS binding to $\alpha_1\beta_2\gamma_2$ or α_1(K278M)$\beta_2\gamma_2$ $GABA_A$ receptors were 31 and 39 min respectively. Binding to mutant receptors at equilibrium was 47% of binding to wild type receptors (3098 and 6656 fmol mg^{-1} protein respectively). **B**. The K278M mutation reduced benzodiazepine binding. HEK membranes containing wild-type or mutant receptors were incubated in a saturating concentration (50 nM) of [^{3}H]Flunitrazepam for 90 mins. Specific [^{3}H]flunitrazepam binding to wild-type and mutant receptors was 677 and 290 fmol mg^{-1} protein respectively.

Supplemental Figure 3



The K289M epilepsy mutation reduces spontaneous, GABA-independent gating when transiently expressed in HEK293 cells. **A.** Spontaneous leak currents recorded from recombinant $\alpha1\beta2\gamma2$ receptors are inhibited by picrotoxin (100 μM) in the absence of GABA (top panel). GABA (1 mM) evoked a typical whole-cell response in the same experiment, demonstrating the presence of functional $GABA_A$ receptors. **B**. Mutant $\alpha1(K278M)\beta2\gamma2$ receptors have diminished spontaneous gating, as evident by the lack of current blockade in the presence of picrotoxin (100 μM). Mutant receptors also exhibit a typical whole cell response to GABA (1 mM). **C**. Graphical summary of spontaneous leak current recorded from different $GABA_A$ receptor subtypes. Spontaneous current is expressed as a percentage of whole cell current evoked by GABA. Mutant subunits containing the K289M substitution caused a reduction in spontaneous current compared to wild type receptors. Data are represented as mean ± S.E.M. *$p < 0.05$ compared to $\alpha1\beta2\gamma2$ by Student's *t*-test.