

Supplementary Figure 1. Detection of surface recombinant GABRG2 using anti-GFP staining on live neurons. Neurons were either transfected with GFP only (A) or co-transfected with GFP and GFP-tagged recombinant GABRG2 (B). An anti-GFP antibody (red) was applied on living neurons at 4°C (see Methods) prior to permeabilization and application of another GFP-antibody to recognize cytoplasmic GFP (green). No signal was detected upon live staining of neurons transfected with GFP only. In contrast, punctuate staining was observed in neurons co-transfected with recombinant $\gamma 2$, suggesting this staining only revealed the extracellularly exposed GFP tag. Scale: 5 μm . (C) Overexpression of recombinant $\gamma 2$ did not significantly affect the gross morphology or electrophysiological properties of transfected neurons. Neurons transfected with wild-type vs. K289M or R43Q mutant GABRG2 showed no significant difference in mean number of primary dendrites ($p=0.8$ and 0.9 , respectively), membrane resistance ($p=0.5$ and 0.1 , respectively) or membrane capacitance ($p=0.7$ and 0.5 , respectively). $n=37$ – 61 neurons for primary dendrite counts, and 14 – 24 for membrane properties.

Supplementary Figure 2. Reduced colocalization of recombinant and endogenous $\gamma 2$ in neurons expressing R43Q GABRG2. (A) Recombinant $\gamma 2$ clusters (red) colocalized (arrowheads) with endogenous $\gamma 2$ -immunopositive clusters (green) in neurons expressing WT or K289M, but not R43Q recombinant GABRG2. Note that, in neurons expressing WT or K289M GABRG2, not all clusters immunopositive for $\gamma 2$ show GFP immunoreactivity, possibly reflecting endogenous $\gamma 2$ clusters lacking recombinant $\gamma 2$. As for $\alpha 1$ and $\alpha 5$, R43Q $\gamma 2$ clusters were often intercalated between endogenous $\gamma 2$ clusters. Scale: 5 μm . (B) Endogenous $\gamma 2$ surface expression was measured in dendrites outlined from post-fixation, anti-GFP stained neurons transfected with either WT, K289M or R43Q recombinant GABRG2 (16 to 22 cells each). Cumulative histograms show no difference in the anti- $\gamma 2$ immunofluorescence detected in either condition (Kolmogorov-Smirnov test, $p>0.8$). (C) Mean pixel intensities averaged from the all cells in each group are also not significantly different in all three conditions ($p>0.8$).

Supplementary Figure 3. Variability of tonic, GABAergic inhibition in cultured hippocampal neurons. Untransfected neurons were recorded after 3 weeks in culture with a CsCl-based internal

solution (estimated $E_{Cl} = -1$ mV). (A) and (B) show recordings from two different neurons before (control) and after >5 minutes of bicuculline methochloride (20 μ M) application. The left panels show probability density functions of baseline current measured from 10.000 to 60.000 sample points under each condition (as in Glykys & Mody, 2006). The values of mean baseline currents are indicated. (C) Data from 4 independent recordings showing the variability of bicuculline-sensitive tonic currents in cultured neurons (CV=0.43).