Table S1. Proteins Associated with GABA$_A$Rγ2, Related to Figure 2

Proteins associated with HFY-GABA$_A$Rγ2 were purified using His and YFP tags from four independent experiments. Age- and sex-matched *Thy1-HFY-GABA$_A$Rγ2* (TG) and wildtype (WT) mice were used for comparison to remove potential false positives. Proteins listed in this table have passed the following criteria: detected in at least two of four experiments for a protein exclusively found in TG sample; peptide spectrum match (PSM) ratio ≥ 6:1 for a protein detected in both TG and WT samples. PSM listed here are cumulative total PSM from four experiments. Protein categories were defined by manual curation from PubMed and GeneCard.

Accession	Gene symbol	Protein name	WT total PSM	TG total PSM
CON_GFP	GFP	Green fluorescent protein	0	70
Known associated proteins				
IPI00113772.1	Gabra1	Gamma-aminobutyric acid receptor subunit alpha-1	0	42
IPI00110598.1	Gabra2	Gamma-aminobutyric acid receptor subunit alpha-2	0	15
IPI00110601.3	Gabra3	Gamma-aminobutyric acid receptor subunit alpha-3	0	24
IPI00221880.1	Gabra5	Gamma-aminobutyric acid receptor subunit alpha-5	0	9
IPI00119283.1	Gabrb1	Gamma-aminobutyric acid receptor subunit beta-1	0	36
IPI00323554.3	Gabrb2	Gamma-aminobutyric acid receptor subunit beta-2	0	68
IPI00130546.1	Gabrb3	Gamma-aminobutyric acid receptor subunit beta-3	0	45
IPI00131369.1	Gabrg2	Isoform 2L of Gamma-aminobutyric acid receptor subunit gamma-2	0	60
IPI00223325.5	Gabbr1	Isoform 1A of Gamma-aminobutyric acid type B receptor subunit 1	0	4
IPI00816946.1	Gphn	Gephyrin isoform 1	1	8
IPI00468605.4	Nlgn2	Neuroligin-2	1	13
Protein folding and trafficking				
IPI00187430.3	Rab2b	Ras-related protein Rab-2B	1	6
IPI00653744.1	Lrrc59	Leucine rich repeat containing 59	0	2
IPI00133119.1	Rer1	Retention In Endoplasmic Reticulum Sorting Receptor 1	0	2
IPI00857758.1	Tmub1	Transmembrane and ubiquitin-like domain containing 1	0	3
IPI00123342.4	Hyou1	Hypoxia up-regulated protein 1	1	23
IPI00676130.1	Tmx4	Thioredoxin domain containing 13	0	4
Ion channels				
IPI00331064.7	Cacna1e	Calcium channel, voltage-dependent, R type, alpha 1E subunit	0	2
IPI00761641.1	Scn2a1	Sodium channel, voltage-gated, type II, alpha 1	0	3
Enzyme activities				
IPI00270326.1	Psmc2	Proteasome 26S subunit, ATPase 2	0	2
IPI00322145.10	Ptplad1	Protein tyrosine phosphatase-like protein PTPLAD1	0	2
IPI00309322.3	Hmox2	Heme oxygenase 2	1	7
IPI00119945.1	Nit2	Omega-amidase NIT2	0	2

IPI00121341.1	Tmx1	Thioredoxin-related transmembrane protein 1	0	4
IPI00918076.1	Agpat5	1-Acylglycerol-3-Phosphate O-Acyltransferase 5	0	2
IPI00877337.1	Dhrs7b	Isoform 2 of Dehydrogenase/reductase SDR family member 7B	0	3
Mitochondria and associated				
IPI00225254.6	Mtx2	Metaxin-2	0	6
IPI00420734.1	Mff	Isoform 1 of Mitochondrial fission factor	0	4
IPI00131896.1	Brp44	Brain protein 44	0	2
IPI00857677.1	Mrpl3	Mitochondrial Ribosomal Protein L3	0	3
IPI00230715.5	Ndufa13	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 13	0	3
IPI00655156.3	Ccdc109a	Isoform 1 of Coiled-coil domain-containing protein 109A	0	7
Miscellaneous				
IPI00816859.1	Ccdc127	Isoform 2 of Coiled-coil domain-containing protein 127	0	3
IPI00351206.5	Pgrmc2	Membrane-associated progesterone receptor component 2	0	3
IPI00828496.2	Emd	Emerin	0	5
IPI00125310.1	C1qa	Complement C1q subcomponent subunit A	1	9
IPI00124470.1	C1qc	Complement C1q subcomponent subunit C	0	6
IPI00266836.1	1810026J23Rik	RIKEN cDNA 1810026J23 gene	0	3
IPI00222697.1	Syngr1	Isoform 1B of Synaptogyrin-1	1	9
IPI00881478.1	Ncald	Neurocalcin delta	0	2
IPI00882228.1	Tfg	Trk-fused gene	1	6
IPI00311764.6	BC007180	BC007180 protein	0	2
IPI00471120.2	Itm2c	Integral membrane protein 2C	0	2
IPI00122399.1	Glg1	Golgi apparatus protein 1	1	8
IPI00121627.3	Clptm1	Cleft lip and palate transmembrane protein 1	0	3
IPI00225244.1	Tusc3	Tumor suppressor candidate 3	0	2
IPI00885293.1	Zcwpw1	Zinc Finger CW-Type And PWWP Domain Containing 1	0	3
IPI00886162.1	Ncln	Nicalin	0	2
IPI00755692.1	Lca5	LCA5, lebercilin	0	2
IPI00668429.1	Dsp	Desmoplakin isoform 2	0	2

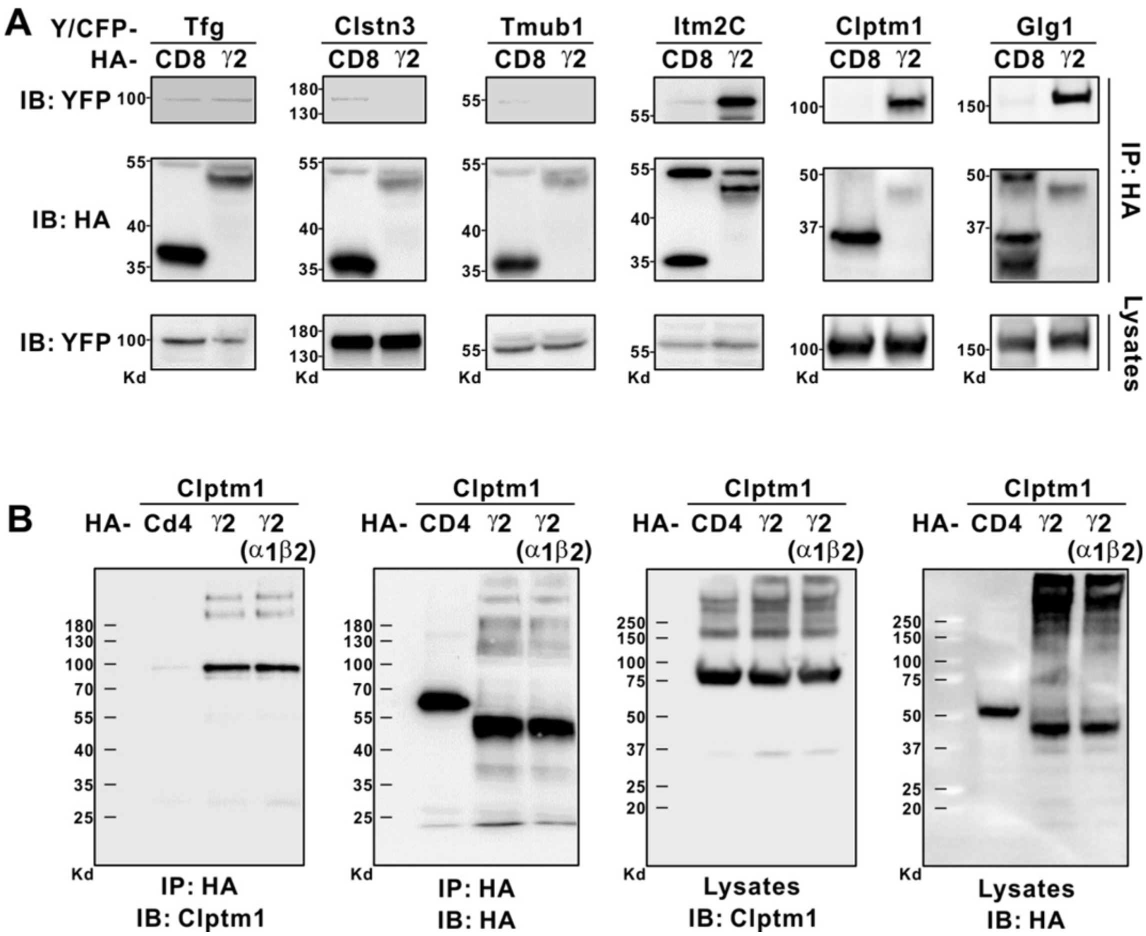
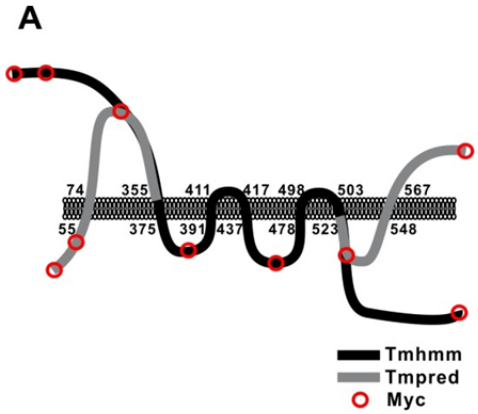


Figure S1. Clptm1, Itm2C, and Glg1 Associate with GABA$_A$R γ2, Related to Figure 2
(A) HEK293 cells were transfected with YFP- or CFP-tagged Trk-fused gene (Tfg), calsyntenin-3 (Clstn3), Transmembrane and ubiquitin-like domain containing 1 (Tmub1), Itm2C, Clptm1, or Glg1 and HA-tagged GABA$_A$R γ2 or CD8 as a negative control. The candidates were chosen from Table S1 except Clstn3 which was isolated from transgenic and not wild type mice from one experiment and had a previously identified functional role in inhibitory transmission. Only Itm2C, Clptm1, and Glg1 were specifically co-immunoprecipitated with GABA$_A$R γ2.
(B) Full Western Blot images of Figure 2B Clptm1.



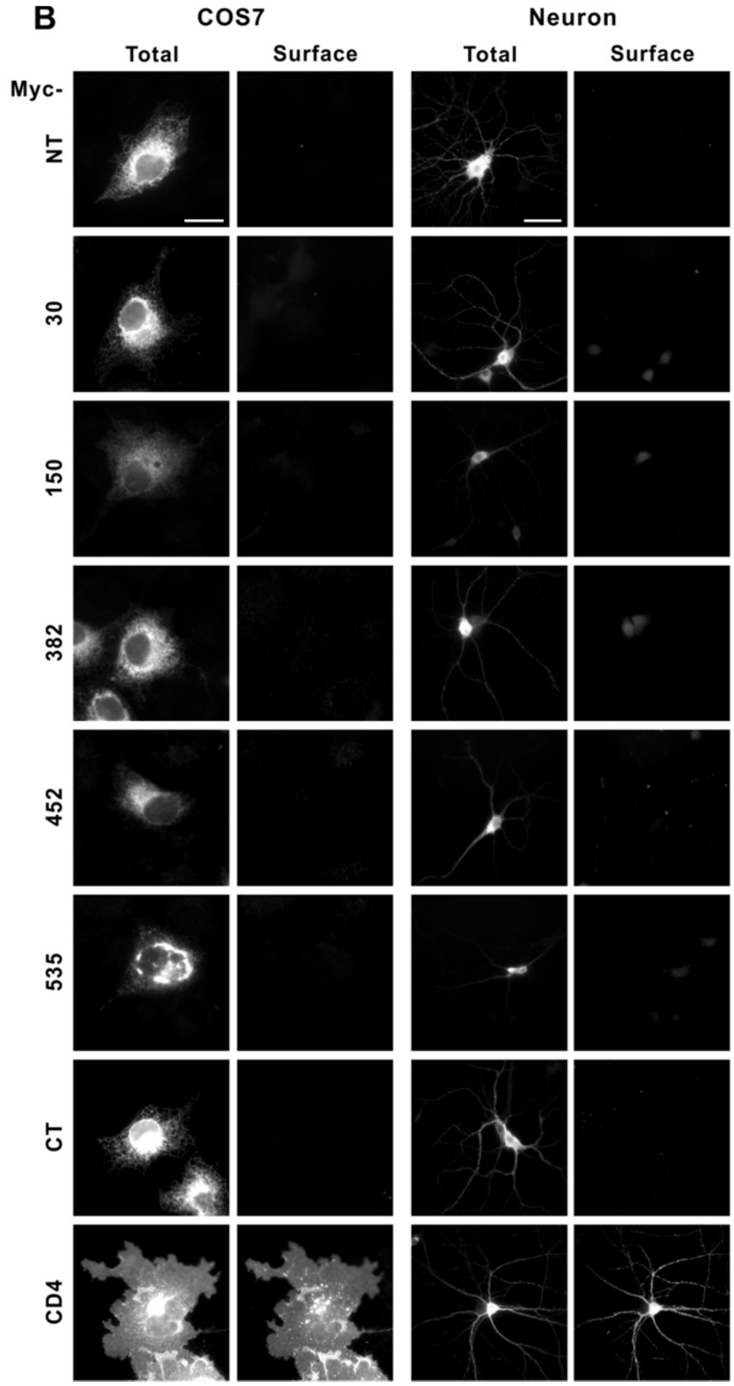


Figure S2. Clptm1 Cannot be Detected on the Cell Surface, Related to Figure 3

(A) The predicted topology of Clptm1 is shown with 5-7 transmembrane domains depending on the prediction program.

(B) A Myc tag was added at the N-terminus (NT), amino acid 30, 150, 382, 452, 535, or C-terminus (CT) of Clptm1 as diagrammed on the predicted structure in panel A. Surface and total Clptm1 were detected using immunostaining in non-permeablized and permeablized conditions, respectively. Intracellular pools were observed but no Myc-Clptm1 was detected on the surface of transfected COS7 cells or cultured hippocampal neurons. Scale bars represent 20 μm in COS7 cells and 50 μm in neurons.

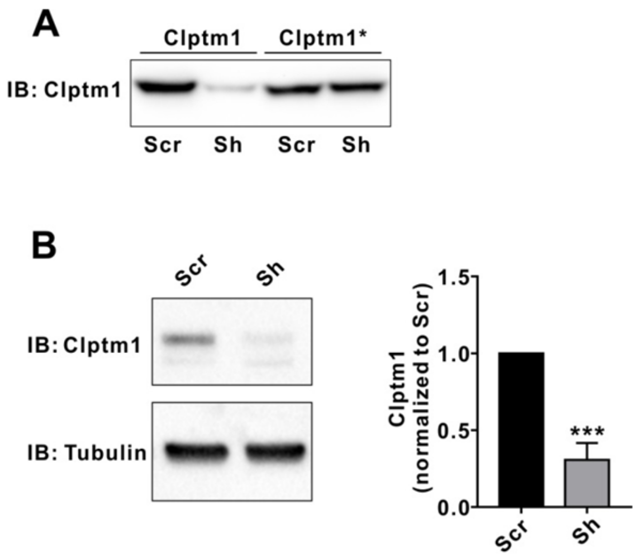


Figure S3. Verification of Clptm1 Knockdown, Related to Figure 5

(A) HEK293 cells were transfected with Clptm1 or RNAi-resistant Clptm1* and shClptm1 (Sh) or shScramble (Scr) as control. Western blotting showed shClptm1 mediated knockdown and verified RNAi resistance of Clptm1*.

(B) AAV vectors expressing shScramble (Scr) or shClptm1 (Sh) were added to cultured cortical neurons at 2 DIV, and cell lysates were collected at 14 DIV. The expression of native Clptm1 was significantly reduced by shClptm1 compared with shScramble. Results are expressed as mean ± SEM. n=3, *** $p < 0.001$, t-test.

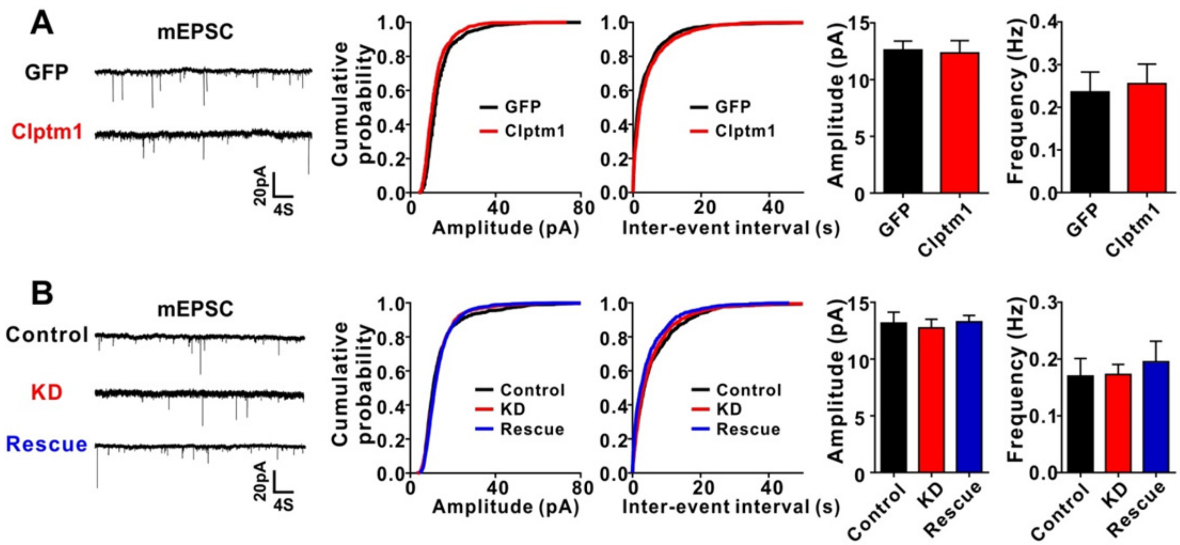


Figure S4. Modulation of Clptm1 Expression Does Not Affect Excitatory Synaptic Transmission *In Vivo*, Related to Figure 6

Neonatal mice were injected with the same AAV vectors as described in Figure 6. mEPSC recordings were performed at P14-17.

(A) Recordings were collected from visualized identified GFP or YFP positive CA1 pyramidal neurons. Overexpression of Clptm1 did not change mEPSC amplitude or frequency. n=22-23 cells from 2 mice each group.

(B) Recordings were collected from visualized identified Tdtomato and GFP or YFP dual positive CA1 pyramidal neurons. Knockdown of Clptm1 did not change mEPSC amplitude or frequency. n=25-27 cells from 2 mice each group.

Results are expressed as mean ± SEM.

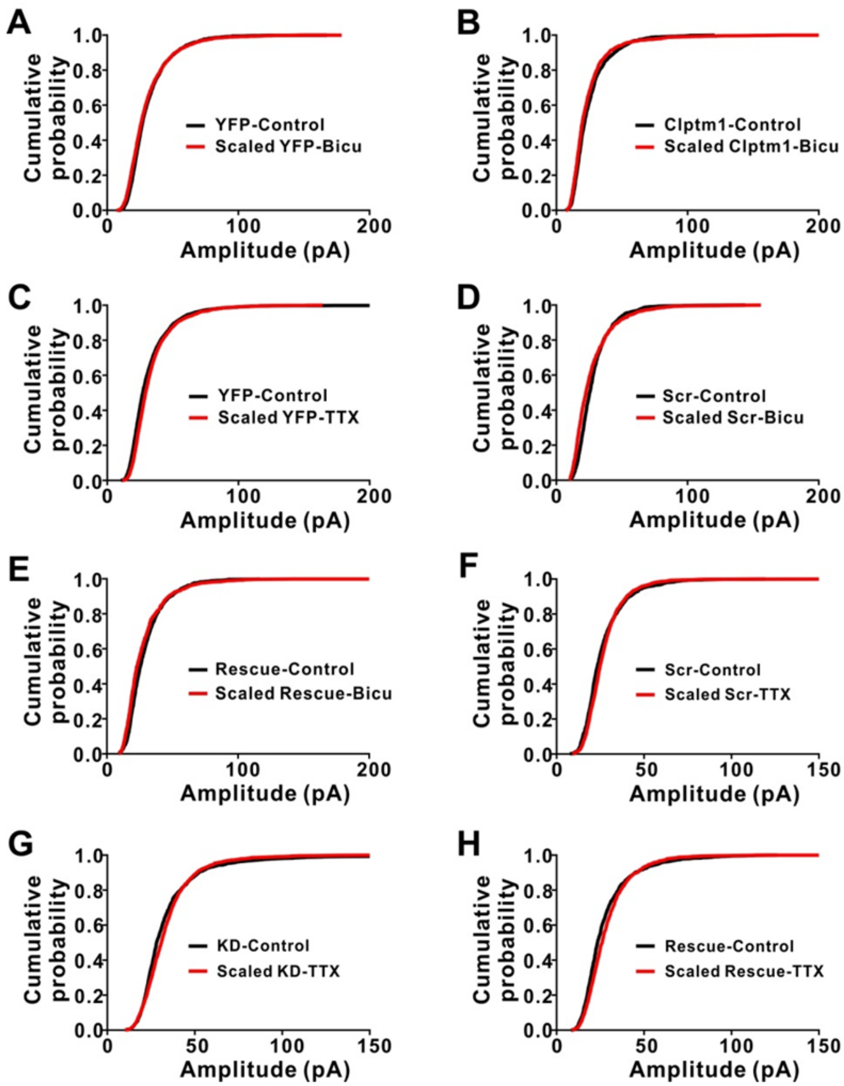


Figure S5. Bicuculline and TTX Induce Multiplicative Scaling of GABAergic Synaptic Strength, Related to Figure 7

Multiplicative scaling up of mIPSC amplitude was induced by bicuculline. The amplitude of mIPSCs recorded from bicuculline treatment groups was scaled by dividing by a factor of 1.45 (A, neurons transfected with YFP), 1.61 (B, neurons transfected withYFP-p2a-Clptm1), 1.54 (D, neurons transfected with shScramble-Tdtomato and YFP), or 1.53 (E, neurons transfected with shClptm1-Tdtomato and YFP-p2a-Clptm1*), and compared to the corresponding control group. K-S tests showed no significant differences between control and scaled groups.
Multiplicative scaling down of mIPSC amplitude was induced by TTX. The amplitude of mIPSCs recorded from TTX treatment groups was scaled by dividing by a factor of 0.80 (C, neurons transfected with YFP), 0.81 (F, neurons transfected with shScramble-Tdtomato and YFP), 0.70 (G, neurons transfected with shClptm1-Tdtomato and YFP), 0.79 (H, neurons transfected with shClptm1-Tdtomato and YFP-p2a-Clptm1*), and compared to the corresponding control group. K-S tests showed no significant differences between control and scaled groups.

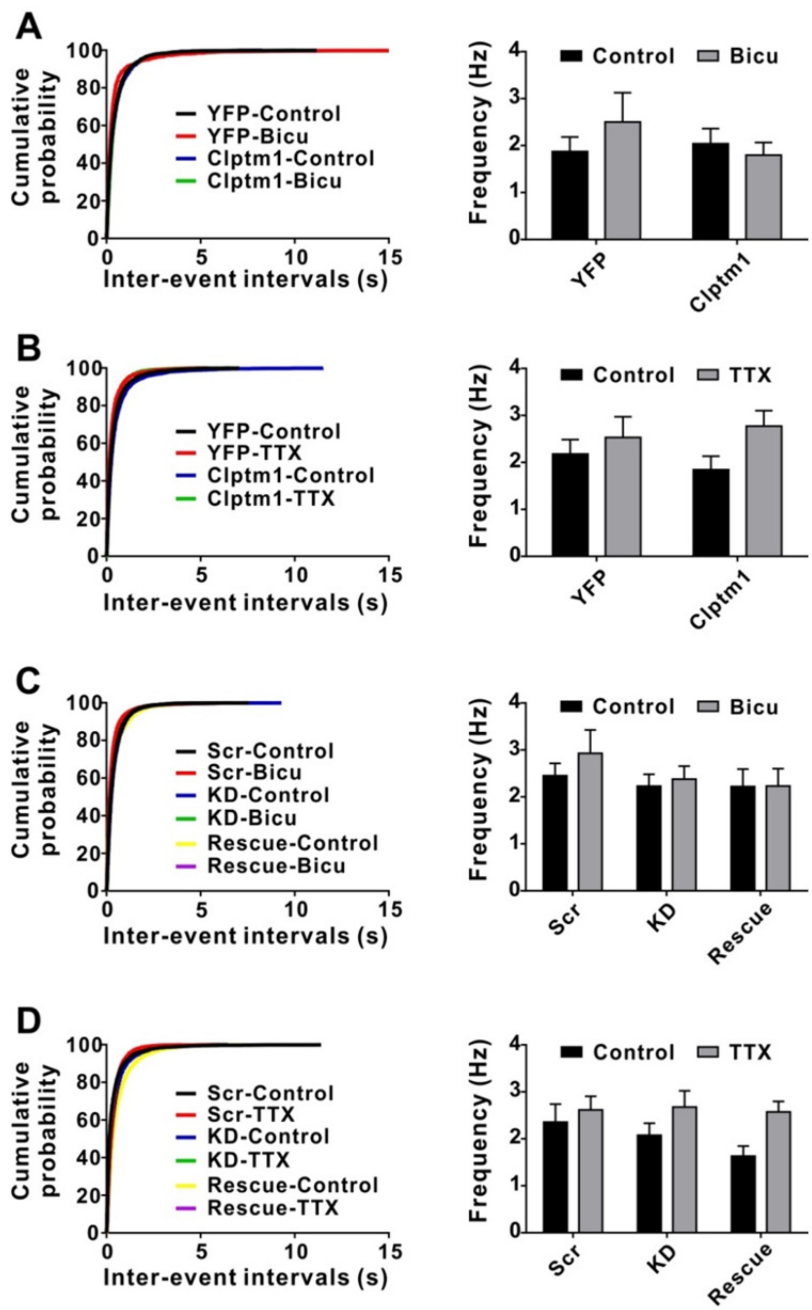


Figure S6. Clptm1-modulated Inhibitory Synaptic Homeostasis Does Not Involve Changes in mIPSC Frequency, Related to Figure 7

Cultured neurons were transfected and mIPSCs recorded as described in Figure 7. Transfection groups were Clptm1-p2a-YFP (Clptm1) compared with YFP control (A and B) or shClptm1-Tdtomato and YFP for knockdown (KD) compared with shScramble-Tdtomato and YFP (Scr) or shClptm1-Tdtomato and YFP-p2a-Clptm1* (rescue) controls (C and D). Significant changes in mIPSC frequency were not detected among groups upon treatment with bicuculline (A and C) or TTX (B and D) except for panel (D) there was a significant effect of TTX ($p<0.05$ two-way ANOVA) but no significant effect of Clptm1 knockdown nor significant differences among groups in posthoc pairwise Holm-Sidak tests.