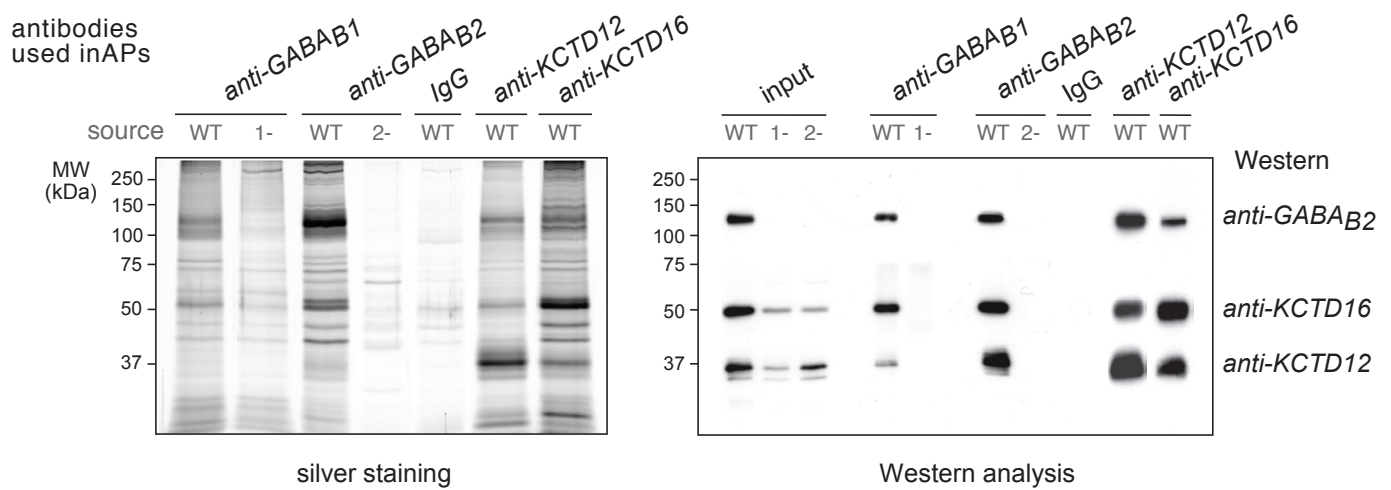
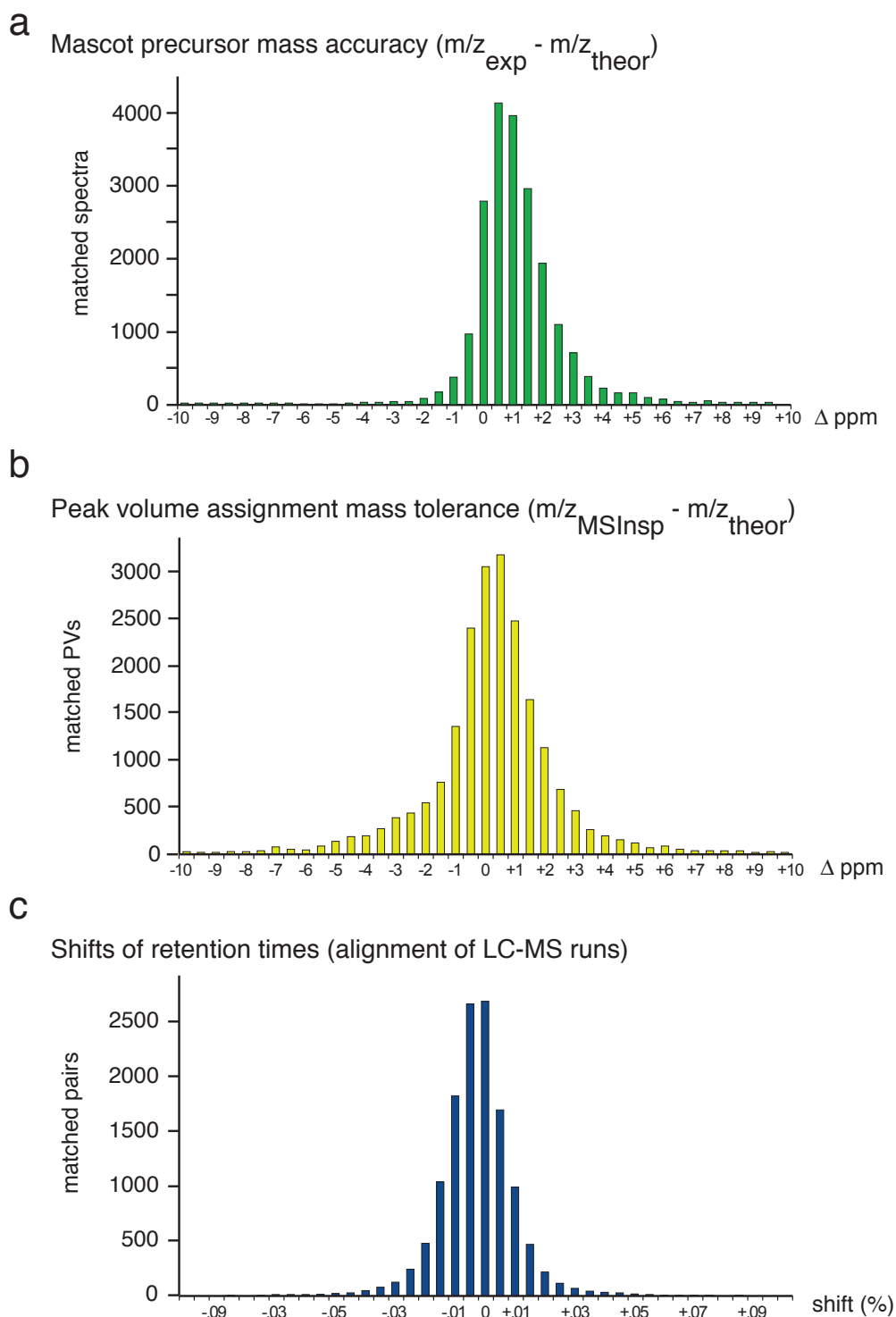


## SUPPLEMENTARY INFORMATION

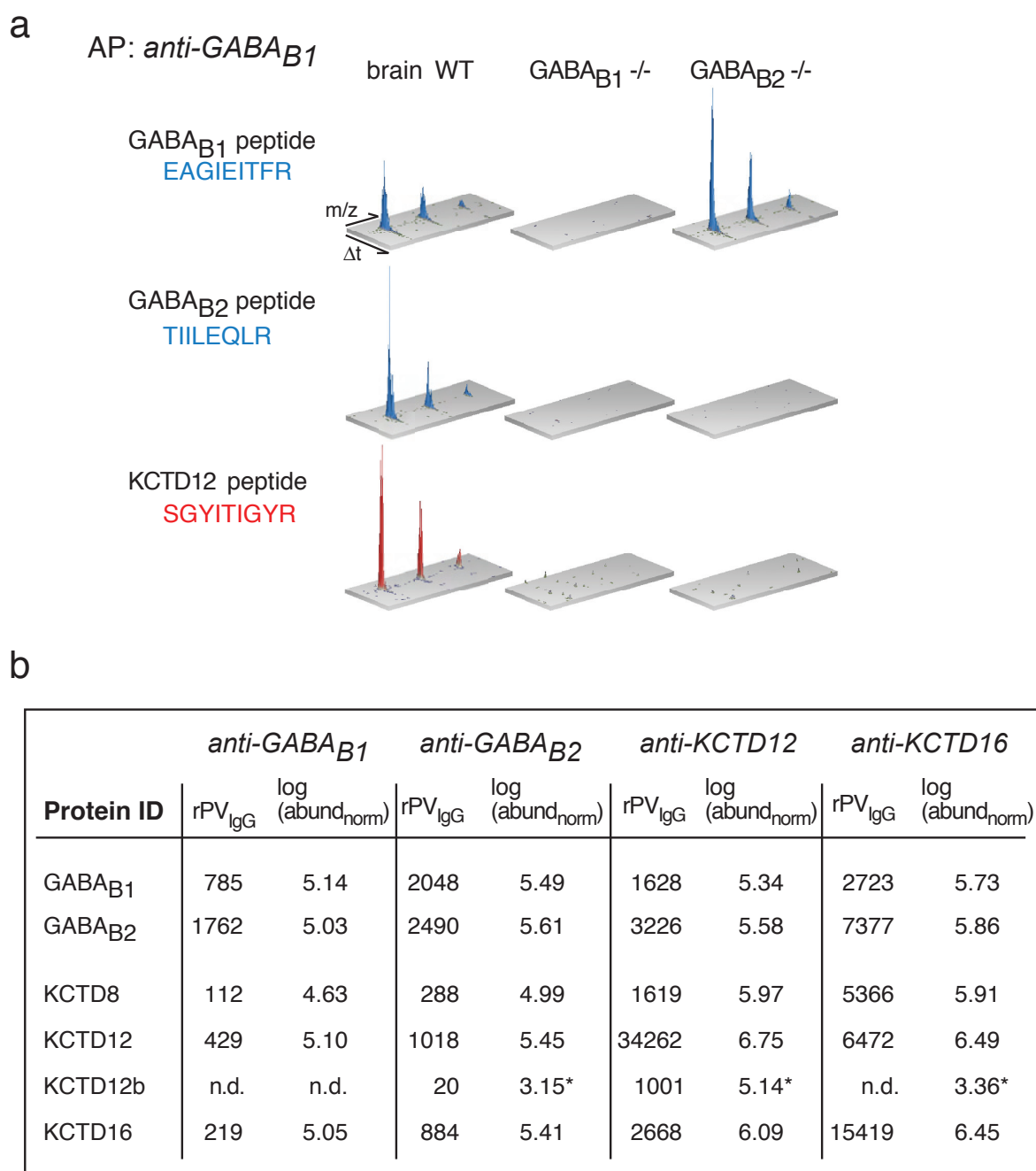
**Supplementary Figure 1****Gel separations of APs with *anti-GABA<sub>B</sub>* and *anti-KCTD* antibodies.**

Left panel: Silver-stained SDS-PAGE separations of APs with the indicated antibodies from wild-type and GABA<sub>B1</sub><sup>-/-</sup> or GABA<sub>B2</sub><sup>-/-</sup> mice. Right panel: SDS-PAGE separation of input and eluate of the APs shown on the left Western blotted with the indicated antibodies.



### Supplementary Figure 2

**Accuracy of LC-MS/MS measurements** (over all nano-LC MS/MS runs performed). **a**, Histogram summarizing the error distribution of  $m/z$  values ( $m/z_{\text{exp}}$  is determined by Excalibur as precursor ion mass used for Mascot-based data base searches,  $m/z_{\text{theor}}$  is mass of the peptide retrieved by Mascot in data base searches). **b**, Histogram as in (a) with  $m/z_{\text{MSInsp}}$  referring to the  $m/z$  value determined by MSInspect from consecutive LC-MS spectra. **c**, Distribution of relative shifts in retention time between peptide signals (LC-MS feature  $m/z$ ) in matched pairs of datasets. Note that all three distributions are well within the thresholds for peptide mass tolerance ( $\pm 10$  ppm) and retention time shifts (10%) used for matching the data sets (see Methods).



### Supplementary Figure 3

**GABA<sub>B</sub> receptors are robustly associated with a subset of KCTD proteins in the rodent brain.** **a**, Peak-volumes (integral of MS signal intensity in the m/z-retention time plane; normal and isotope peaks) of the indicated peptides (z = +2, no modifications) determined from MS-spectra during nano LC-MS/MS analysis of *anti-GABA<sub>B1</sub>* APs from brain membrane fractions of WT and the indicated knock-out mice. All three peptides are representative for the pool of peptides obtained (for the GABA<sub>B</sub> core subunits and the different KCTD proteins, Supplementary Table 2) and used for quantitative evaluation. Note that the KCTD12 peptide was only obtained from WT material. **b**, Relative peak volumes (rPV, see Methods) and logarithmic abundance<sub>norm</sub> values of the GABA<sub>B</sub> receptor core subunits and the KCTD proteins 8, 12, 12b and 16 obtained in APs from rat brain membranes with the indicated antibodies. rPV values > 10 indicate specific copurification; values with asterisks refer to abundance<sub>norm</sub> values corrected for homology between KCTD isoforms 12 and 12b.

GABA<sub>B1a</sub>

1 MLLLLLVPLF LRPLGAGGAQ TPNATSEGCQ I IHPPWEGGI RYRGLTRDQV  
 51 **KAINFLPVDY EIEYVCRGER** EVVGPVKVRKC **LANGSWTDMD TPSRCVTRICS**  
 101 **KSYLTLENGK VFLTGGDLPA** LDGARVEFRC **DPDFHLVGSS RSVCSQGQWS**  
 151 **TPKPHCQVNR** TPHSERRAVY IGALFPMSGG WPGGQACQPA VEMALEDVNS  
 201 **RRDILPDYEL KLIHHDKCD** PGQATKYLYE **LLYNDPIKII LMPGCSVST**  
 251 **LVAEAARMWN LIVLSYGSSS** PALSNRQRF **TFFRTHPSAT LHNPTRVKLF**  
 301 **EKWGWK KIAT IQQTTEVFTS** TLDDLEERVK **EAGIEITFRQ SFFSDPAVPV**  
 351 **KNLKRQDARI IVGLFYETEA** RKVFCEVYKE **RLFGKKYVWF LIGWYADNWF**  
 401 **KTYDPSINCT VEEMTEAVEG** HITTEIVMLN PANTRISISNM **TSQEFVEKLT**  
 451 **KRLKRHPEET GGFQEAPLAY** DAIWALALAL **NKTSGGGGRS GVRLEDFNYN**  
 501 **NQTITDQIYR AMNSSSFEGV** SGHVVDASG **SRMAWTLIEQ LQGGSYKKIG**  
 551 **YYDSTKDDL SSKTKDWIGG** SPPADQTLVI **KTFRFLSQKL FISVSVLSSL**  
 601 **GIVLAVVCLS FNIYNHSHVRY** IQNSQPNLNN **LTAVGCSLAL AAVFPLGLDG**  
 651 **YHIGRSQFPF VCQARLWLLG** LGFSLGYGSM **FTKIWWVHTV FTKKEEKKEW**  
 701 **RKTLEPWKLY ATVGLLVGMD** VLTALAIWQIV **DPLHRTIETF AKEEPKEDID**  
 751 **VSILPQLEHC SSKKMNTWLG** IFYGYKGLLL **LLGIFLAYET KSVSTEKIND**  
 801 **HRAVGMAYN VAVLCLITAP** VTMLSSQOD **AAFAFASLAI VFSSYITLVV**  
 851 **LFVPKMRRLI TRGEWQSETQ** DTMKTGSSSTN **NNEEEKSRLI EKENRELEKI**  
 901 **IAEKEERVSE LRHQLQSRQQ** LRSRHPPTP **PDPSSGGLPRG PSEPPDRLSC**  
 951 **DGSRVHLLYK**

GABA<sub>B2</sub>

1 **MASPPSSGQP RPPPPPPPPA** RLLLPLLLSL **LLWLAPGAWG WTRGAPRPPP**  
 51 **SSPPLSIMGL MPLTKEVAKG** SIGRGLPAV **ELAIEQIRNE SLLRPYFLDL**  
 101 **RLYDTECDNA KGLKAFYDAI** KYGNHLMVF **GGVCPVTSI IAESLQGNL**  
 151 **VQLSFAATTP VLADKKKYPY** **FFRTVPSDNA VNPAILKLLK** HFRWR**RVGTL**  
 201 **TQDQR**FSEV RNDLTGVLYG **EDIEISDTE** FSNDPCTSVK **KLKGNVRII**  
 251 **LGQFDQMAA KVFCFAFEES** MFGSKYQWII **PGWYEPAWWE QVHVEANSSR**  
 301 **CLRRSLLAAM EGYIGVDFEP** LSSKQIKTIS **GKTPQQFERE YNSKRSGVGP**  
 351 **SKFHGYAYDG IWVIAKTLQR** AMETLHASSR **HQRIQDFNYT DHTLGKIILN**  
 401 **AMNETNFFGV TGQVFRNGE** RMGTIK**FTQF** **QDSREVKVGE YNAVADTLEI**  
 451 **INDTIRFQGS EPPKDKTIIL** EQLRKISLPL **YSILSALTIL GMIMASAFLF**  
 501 **FNIKNRNQL IKMSSPYMNN** LIILGGMLSY **ASIFLFGLDG SFVSEKTFET**  
 551 **LCTVRTWILT VGYTTAFGAM** FAKTWRVHAI **FKNVKMKKKI IKDQKLLVIV**  
 601 **GGMLLIDLCI LICWQAVDPL** RRTVERY**SME** **PDPAGRDISI RPLLEHCENT**  
 651 **HMTIWLGIYV AYKGLLMLFG** CFLAWETRV **SIPALNSKY IGMSVYNVGI**  
 701 **MCIIGAASF LTRDQPNVQF** CIVALVIIFC **STITLCLVFEV PKLITLRTNP**  
 751 **DAATQNRFO FTQNKKEDS** KTSTSVTSVN **QASTSRLEGL QSENHRLRMK**  
 801 **ITELDKLEE VTMQLQDTPE** KTTYIK**QNHY** **QELNDILSLG NFTESTDGGK**  
 851 **AILKNHLDQN PQLQWNTTEP** SRTCKDPIED **INSPEHIQR LSLQLPILHH**  
 901 **AYLPSIGVD ASCVSPCVSP** TASPRHR**HVP** **PSFRVMVSGL**



## KCTD8

1 MALKDTGSGG STILPISEMV SASSSPGAPL AAPGPCAPS PFPEVVELNV  
 51 GGQYVYTKHS **TLLSVPDSTL** **ASMFSPSSPR** GGARRRGDLP RDSRAR**FFID**  
 101 **RDGFLEFRYVL** **DYLRDKQLAL** **PEHFPEKERL** **LRAEFFQLT** **DLVKLLSPKV**  
 151 TKQNSLNDEG CQSDLEDNLS QGSSDALLLR **GAAAGAPSSS** **GAHGVSGVVS**  
 201 **GGAPDKRSG** **FTLLGYRGSY** **TTVRDNQADA** **KFRRVARIMV** **CGRIALAKEV**  
 251 **FGDTLNESRD** **PDRQPEKYTS** **RFYLKFTYLE** **QAFDRLSEAG** **FHMVACNSSG**  
 301 **TAAAFVNQYRD** **DKIWSSYTEY** **IFFRPPQKIV** **SPKQEHEDRK** **CDKVTDKGSE**  
 351 SGTSCNELST SSCDSHSEAS TPQDNLVNTQ QAVSQQPNTL TLD RPSRKAP  
 401 **VQWMPDPDR** **RNSELFQSLI** **SKSRETNLSK** **KKVCEKLSVE** **EEMKKCIQDF**  
 451 **KKIHIPDCFP** **ERKRQWQSEL** **LQKYGL**

## KCTD12

1 MALADSTRGL PNGGGGGGGS GSSSSSAEPP LFPDIVELNV GGQYVYTRRC  
 51 **TVVSVPSLL** **WRMFTQQPQ** **ELARDSKGRF** **FLDRDGFLFR** **YILDYLRDLQ**  
 101 **LVLEDFYPER** **SRLQREAEYF** **ELPELVRRIG** **APQQPGPGPP** **PPHSRRGVHK**  
 151 **EGSLGDELLP** **LGAEPEPQE** **GASAGAPSPT** **LDLASRSPSG** **GAAGPLLTSP**  
 201 **QSLDGSRRSG** **YITIGYRGSY** **TIGRDAQADA** **KFRRVARITV** **CGKTSLAKEV**  
 251 **FGDTLNESRD** **PDRPPERYTS** **RYYLKFNFLE** **QAFDKLSESG** **FHMVACSSTG**  
 301 **TCAFASSTDQ** **SEDKIWTSYT** **EYVFCRE**

## KCTD12b

1 MAMPEKSSCV KPTEECSSFP EIIELNVGGQ VYITRYPTLI **SIPGSRLWEM**  
 51 **FSVKNPCSLI** **QDNKGRFFID** **RDGFLEFRYVL** **DYMRDMQVVL** **PDHFPECGR**  
 101 **HREAEIFKLP** **ELAKMLALKM** **NKLNLSIGNDS** **CQIDLDELSP** **SIDTTFNFS**  
 151 **TNSIHISGPD** **NPVVLTAAPG** **SELKAGFIT** **IGYRGSYTLG** **RDSQTDAKER**  
 201 **RVARIMVCGK** **ISLAKEVFGD** **TLNESRD PDR** **PPERYSRY** **LKFTFLEQAF**  
 251 **DKLADAGFHM** **VACNSTGTCT** **VTHDQTDRI** **WTSYTEYVY** **RE**

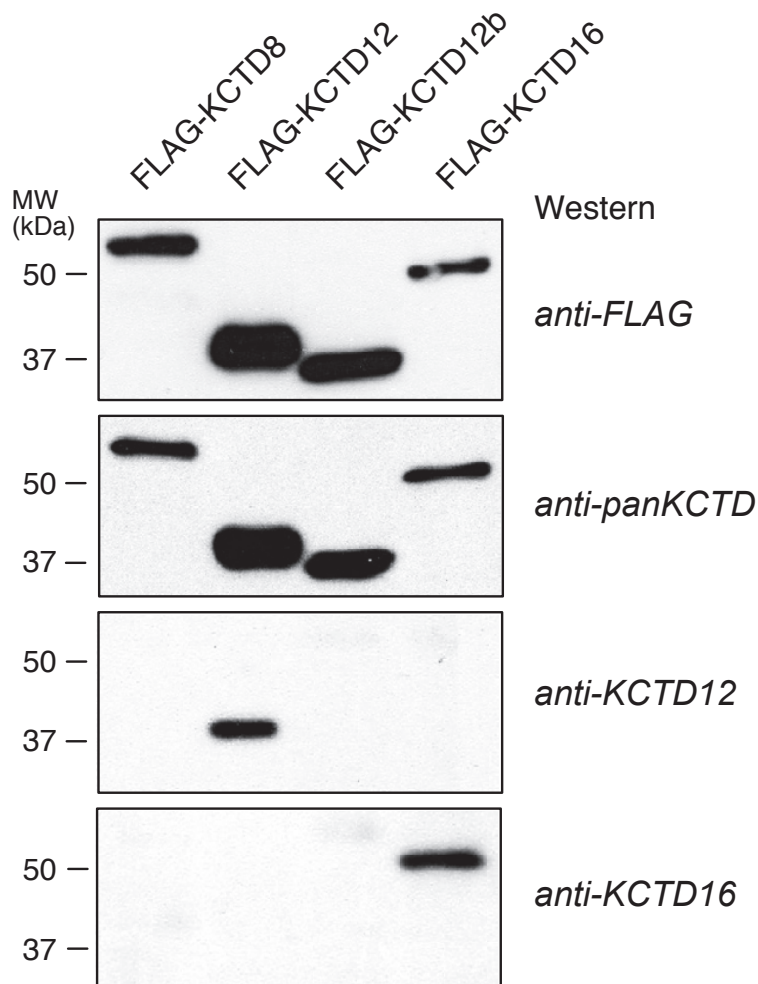
## KCTD16

1 MALSGNCSRY YPRDQGAAPV NSFPEVIELN VGGQYVYFTRH **STLISIPHSL**  
 51 **LWKMFSPKRD** **TANDLAKDSK** **GRFFIDRDGF** **LFYILDYLR** **DRQVVLDPHF**  
 101 **PERGRKREA** **EYFQLPDLVK** **LLAPEEVKQS** **PDEFCHSDFE** **DASQGS DTRI**  
 151 **CPPSSLLPHD** **RKWGFITVGY** **RGSTLGREG** **QADAKFRRVP** **RILVCGRISL**  
 201 **AKEVFGETLN** **ESRDPDRAPE** **RYTSRFLK** **KHLERAFDML** **SECGFHMVAC**  
 251 **NSSVTASFVN** **QYTEDKIWSS** **YTEYVFYREP** **SRWSSSHCDC** **CKNGKGDGK**  
 301 **ESGTSCNDLS** **TSSCDSQSEA** **SSPQETVICG** **PVTRQGNIQT** **LDRPIKGPV**  
 351 **QLIQSEMRR** **KSDLLRTLTS** **GSRESNISSK** **KKAAEKVSI** **EEELEKCIQD**  
 401 **FLKIKIPDRF** **PERKHPWQSE** **LLRKYHL**

## Supplementary Figure 4

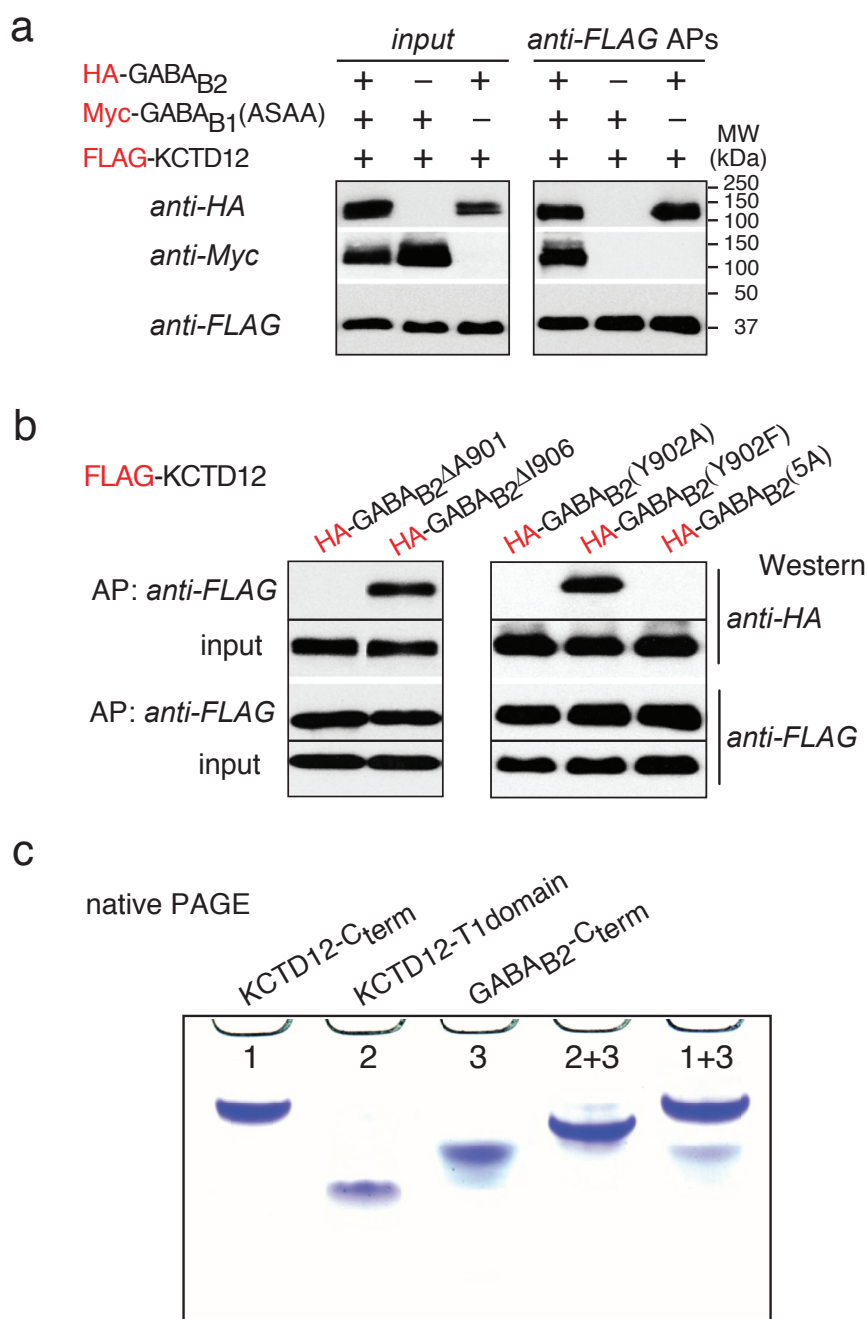
Primary sequences of GABA<sub>B1a</sub>, GABA<sub>B2</sub> and the KCTD proteins 8, 12, 12b and 16. Peptides identified by mass spectrometry are in red; those accessible to but not identified in MS/MS analyses are in black, and peptides not accessible to the MS/MS analyses used are given in grey. The GABA<sub>B2</sub> residue highlighted in green was identified as valine or arginine (polymorphism). The genebank accession numbers of the indicated proteins were: NP\_112290.2 (GABA<sub>B1a</sub>), NP\_113990.1 (GABA<sub>B2</sub>), NP\_001093642.1 (KCTD8), EDM02446.1 (KCTD12), XP\_001065910.1 (and FT174274.1, KCTD12b), XP\_225971.4 (KCTD16).





### Supplementary Figure 6

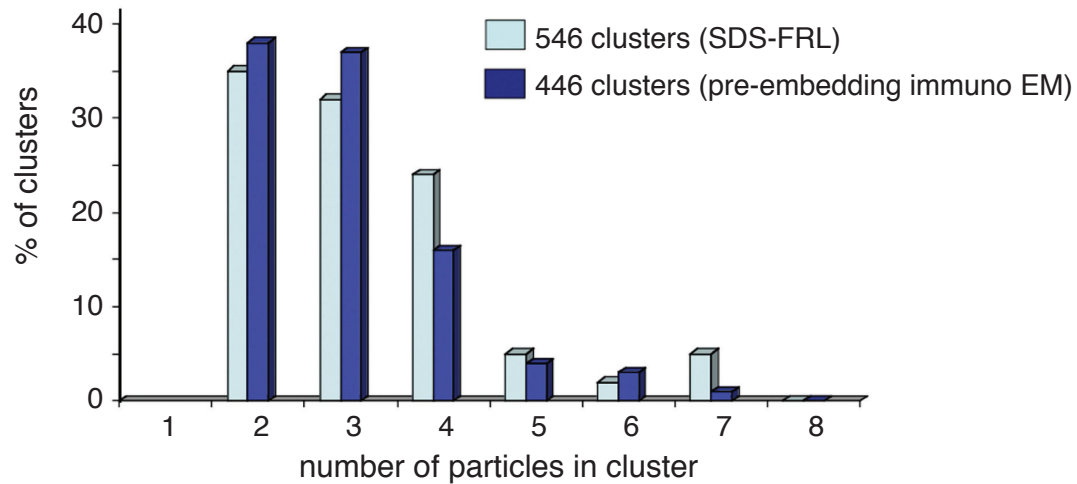
Evaluation of subtype-specificities of the *anti-KCTD12* and *anti-KCTD16* antibodies. SDS-PAGE separated lysates of HEK293 cells expressing the indicated FLAG-tagged KCTD proteins were Western probed with *anti-FLAG*, *anti-KCTD12* and *anti-KCTD16*. An antibody targeting KCTD isoforms 8, 12, 12b and 16 (*anti-panKCTD*) was added for comparison.

**Supplementary Figure 7****Molecular interface of the GABA<sub>B</sub>-KCTD interaction.**

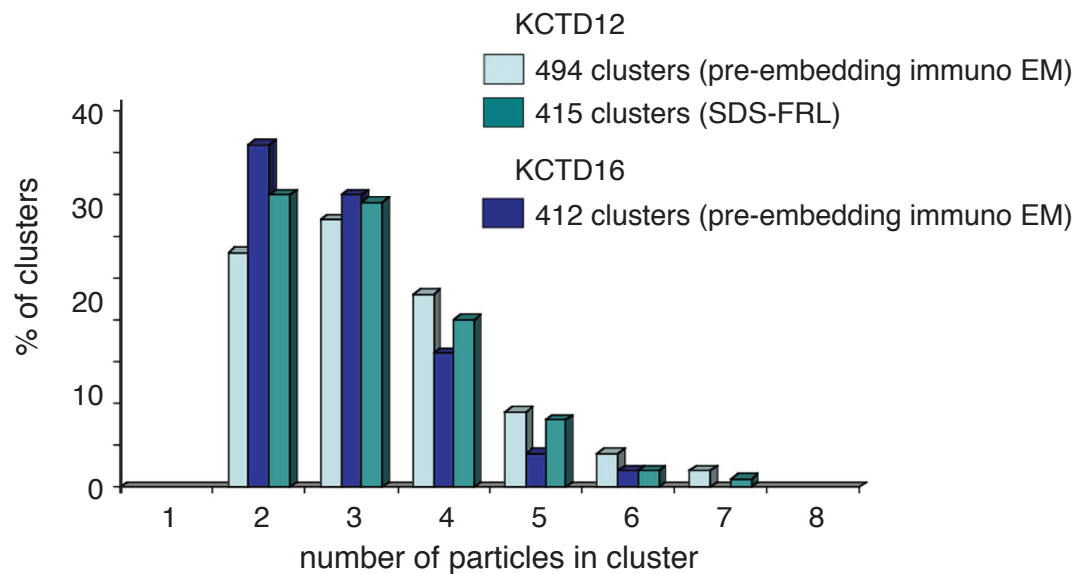
**a**, SDS-PAGE separation of input and eluate of *anti-FLAG* APs Western blotted with the indicated antibodies. Input are membrane fractions from HEK293 cells coexpressing combinations of differentially tagged GABA<sub>B1</sub> (Myc-tag), GABA<sub>B2</sub> (HA-tag) and KCTD12 (FLAG-tag). The GABA<sub>B1</sub>(ASAA) mutant was used to prevent retention of the protein in the endoplasmic reticulum (Pagano et al., 2001). Note the selective interaction of KCTD12 with GABA<sub>B2</sub>. **b**, Experiment as in (a) using FLAG-tagged KCTD12 and HA-tagged C-terminal deletions or point mutations of GABA<sub>B2</sub> as input; in GABA<sub>B2</sub>(5A) amino acids 901-906 are replaced by alanines. Similar results were obtained with KCTDs 8, 12b and 16 (data not shown). **c**, Native PAGE separation of a binding assay using purified T1- and C-terminal domains of KCTD12 (KCTD12-T1, KCTD12-C<sub>term</sub>) and the C-terminus of GABA<sub>B2</sub> (amino acids 849-940). All proteins were expressed in bacteria (see Methods), purified to homogeneity and visualized by Coomassie staining. Note that binding only occurred between KCTD12-T1 and GABA<sub>B2</sub>-C<sub>term</sub>.



a

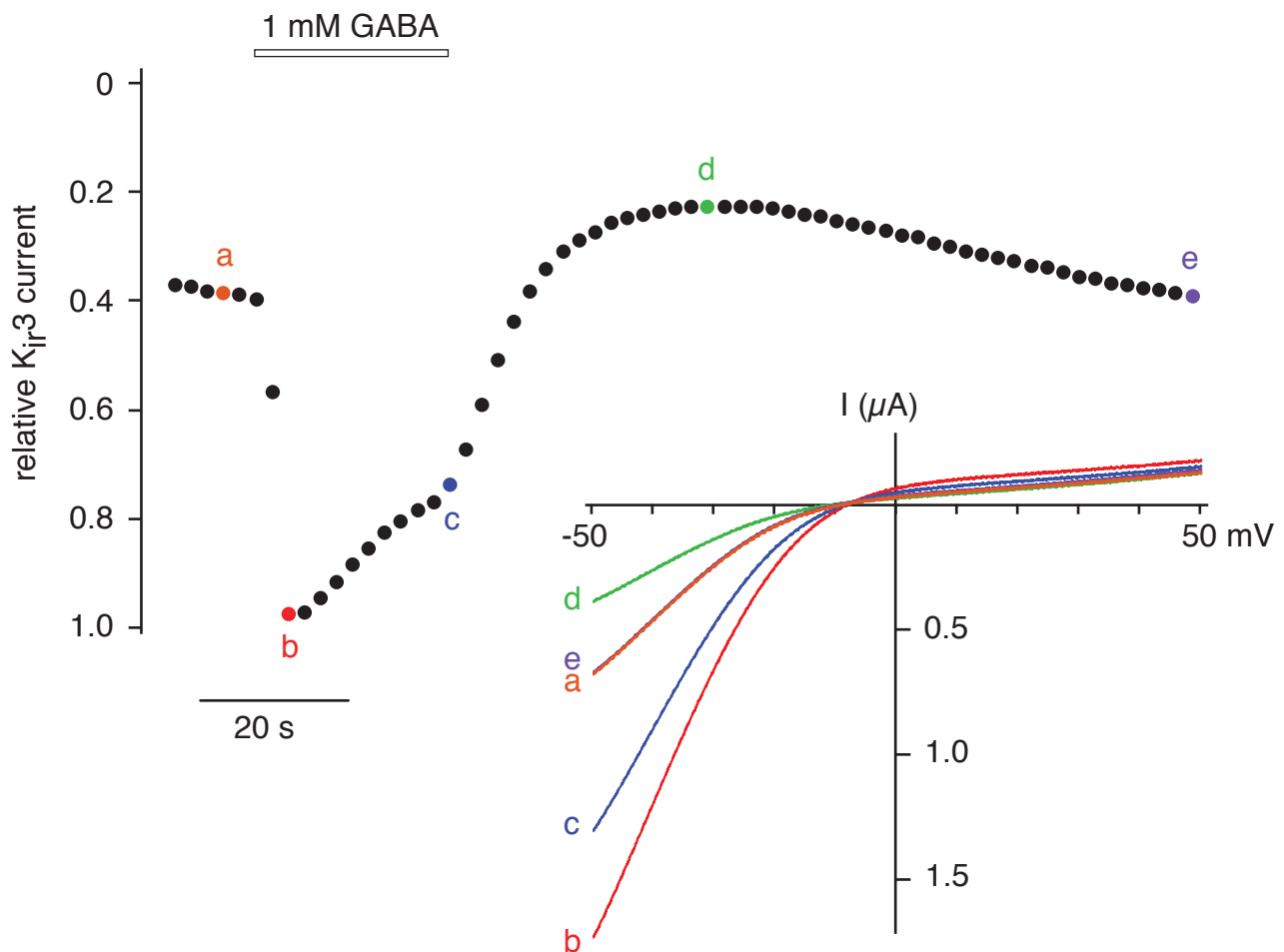
*anti-GABA<sub>B1</sub>* staining

b

*staining with anti-KCTD12, anti-KCTD16***Supplementary Figure 8****Clustering of GABA<sub>B</sub> receptors in dendrites of the hippocampal CA1 region.**

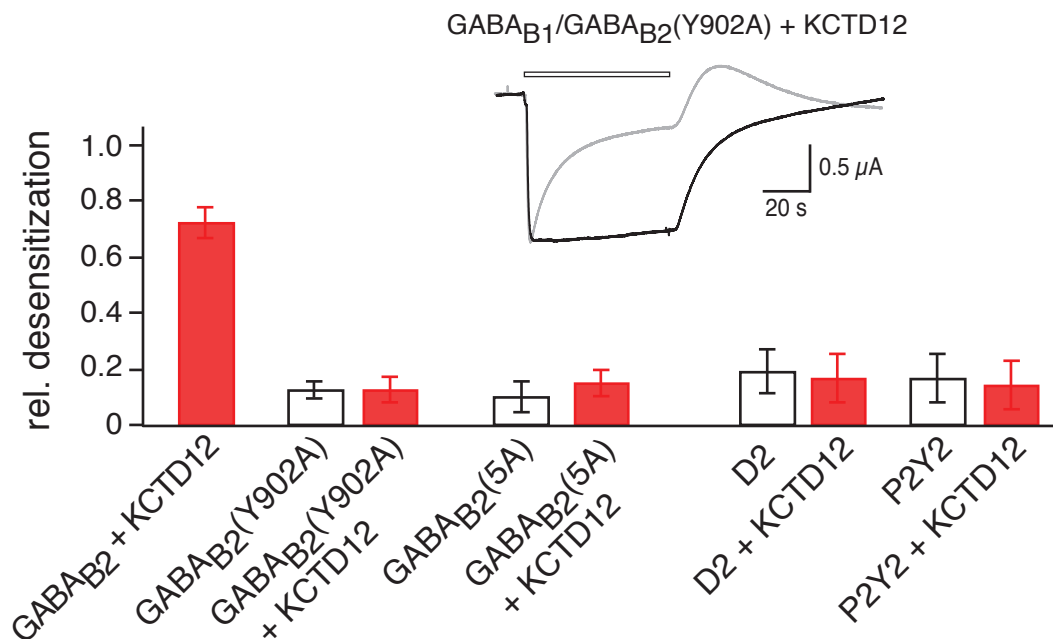
Histograms showing the number of immunogold particles per cluster obtained with the *anti-GABA<sub>B1</sub>* (a), and *anti-KCTD12*, *anti-KCTD16* antibodies (b) in pre-embedding immuno EM and SDS-FRL.

## Xenopus oocytes

 $K_{ir}3.1/3.2 + GABA_B + KCTD12$ **Supplementary Figure 9**

Basal (prior to and after agonist application) and GABA-activated  $K_{ir}3$  currents recorded in oocytes coexpressing  $GABA_B$  and KCTD12 together with  $K_{ir}3.1/3.2$  channels. Currents were elicited by voltage ramps from -50 to 50 mV (2s duration) and the maxima (at -50 mV) were plotted over time. Current traces shown on the right were recorded at the indicated time points. Note that both basal and agonist-triggered currents are mediated by  $K_{ir}3.1/3.2$  channels.

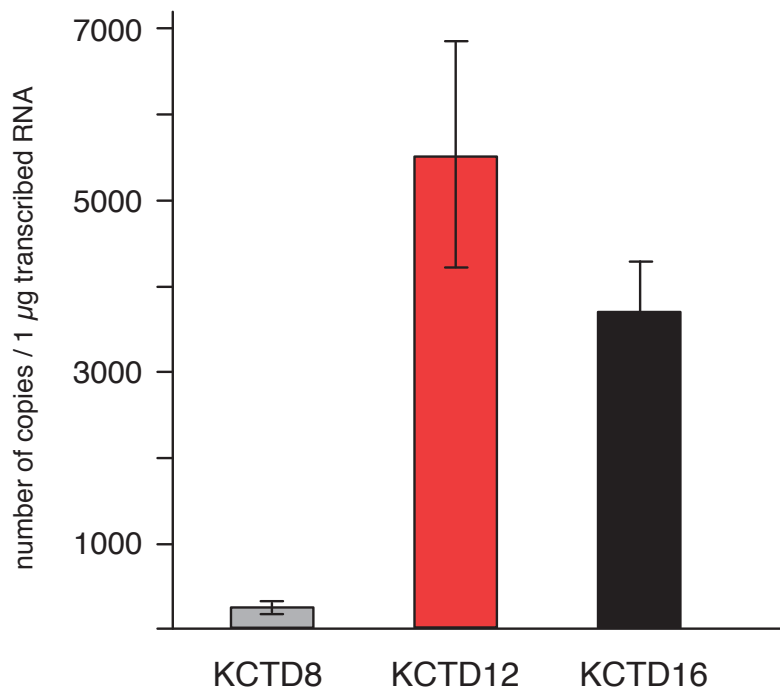
The transient decrease of the basal  $K_{ir}3$  current occurring after removal of the agonist may be envisaged as 'G $\beta\gamma$ '-trapping of 'desensitized'  $GABA_B$  receptors that lasts for as long as the receptors recover from desensitization. Mechanistically, this desensitization likely reflects a ligand-binding dependent transition of the receptor into an 'inactivated' state where further GDP/GTP exchange is precluded thus leading to a decrease of the effector activity - seen as apparent desensitization.



### Supplementary Figure 10

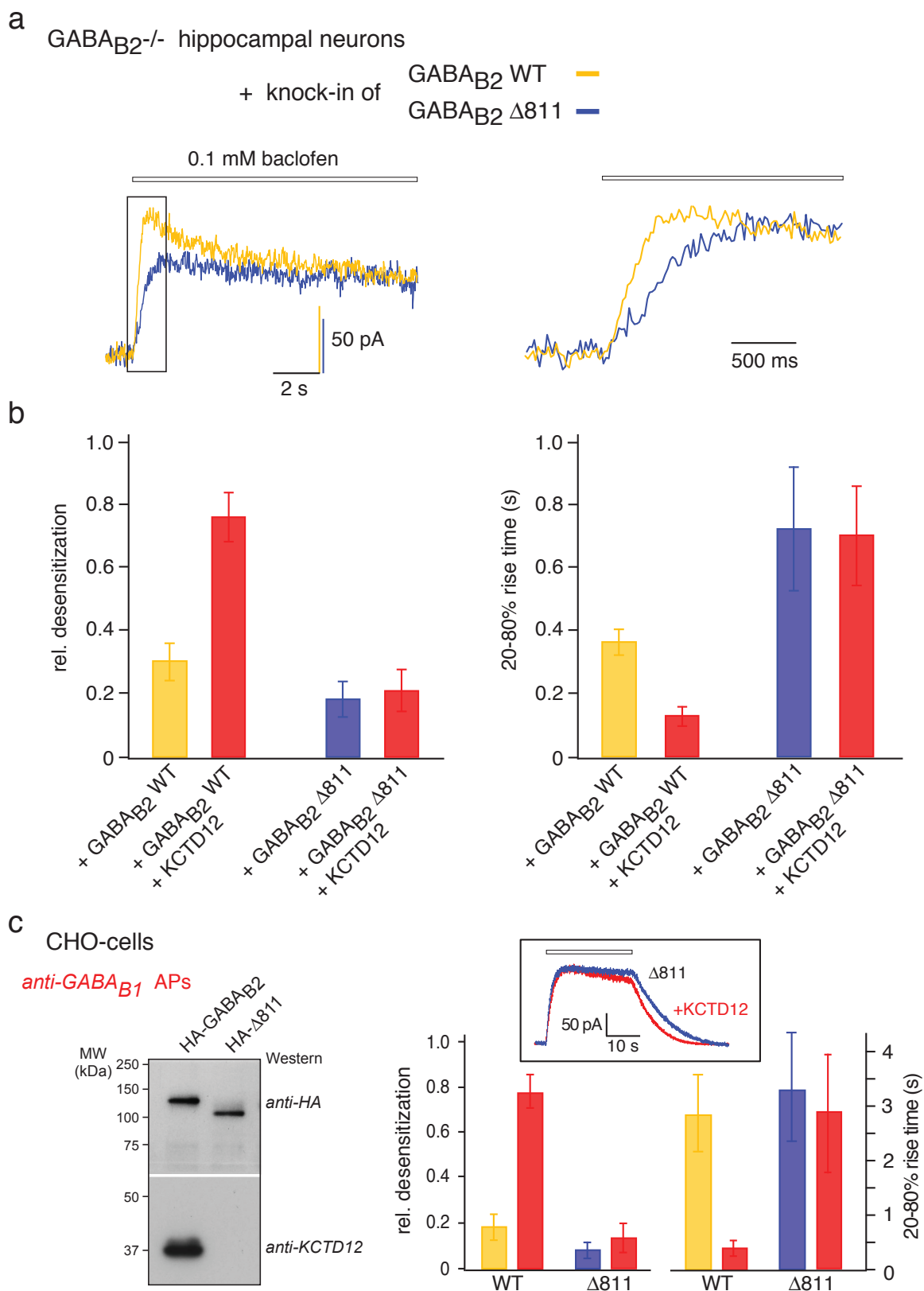
**KCTD effects require association with GABA<sub>B2</sub> and are specific for GABA<sub>B</sub> receptors.**

Relative desensitization of K<sub>i</sub>3.1/3.2 currents determined with the indicated G-protein coupled receptors in the absence and presence of KCTD12. D2, dopamine D2 receptor, P2Y2, metabotropic purine receptor. Inset: Representative K<sub>i</sub>3.1/3.2 current responses activated with GABA<sub>B</sub>-KCTD12 receptors harboring either the GABA<sub>B2</sub> WT (grey) or the GABA<sub>B2</sub>(Y902A) mutant subunit (black). Note that desensitization is abolished in the GABA<sub>B2</sub>(Y902A) mutant precluding assembly with KCTD12.

*hippocampal cultures***Supplementary Figure 11**

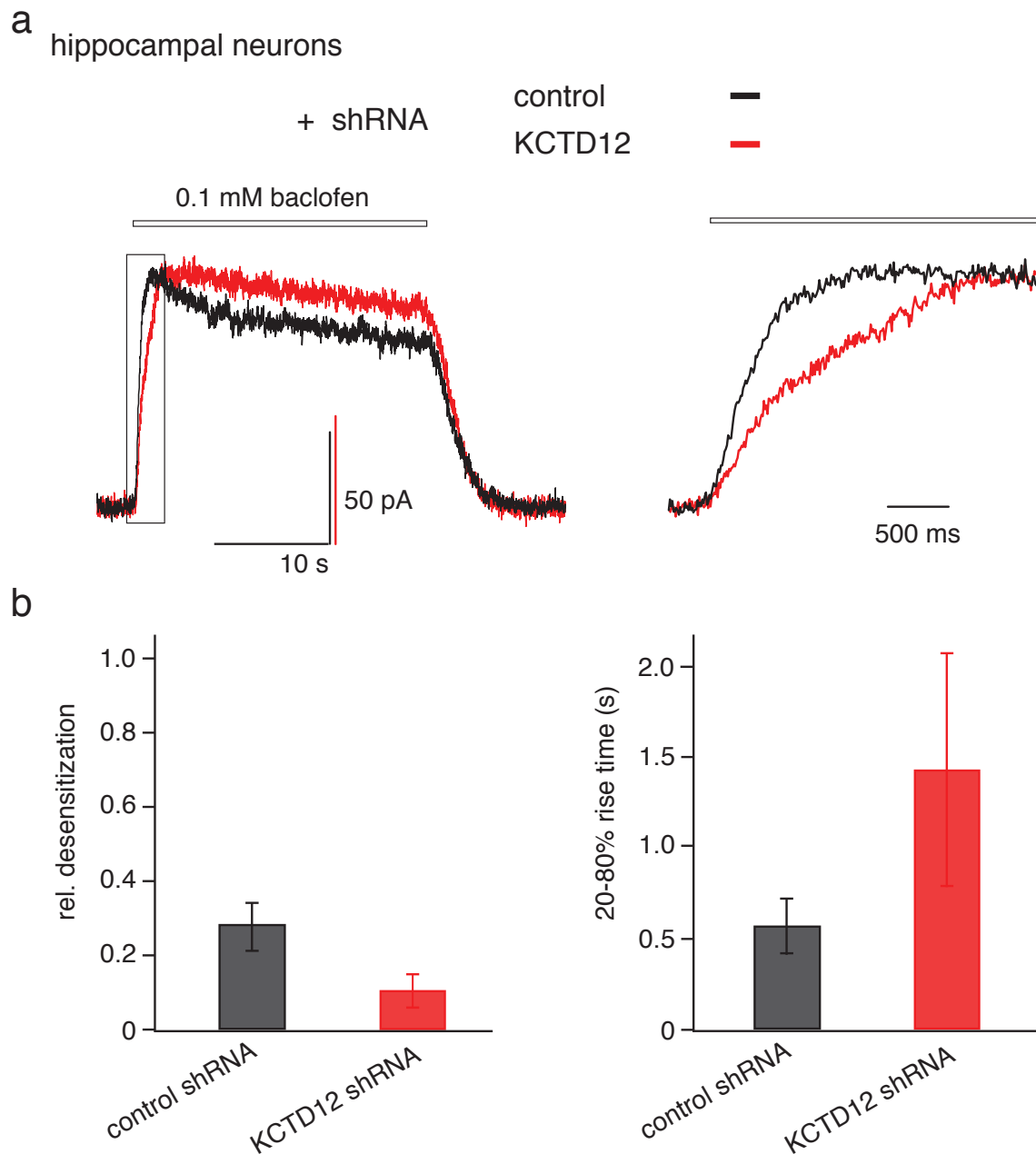
Expression analysis of the indicated KCTD proteins in cultured hippocampal neurons by quantitative PCR. Data are mean  $\pm$  SD of 3 different cultures. qRT-PCR was carried out using the LightCycler and the FastStart DNA Master SybrGreen (Roche, Basel, Switzerland); the primer sequences used were: mKCTD8 (5'-CAACCAGTACCGAGACGACA-3') and (5'-TGTTCTTGCTTGGGTGACACT-3'); mKCTD12 (5'-AGACATTTCTGTACCTTCCACCA-3') and (5'-TTA-TACTAATGGCCCAATCTTTAACA-3'); mKCTD16 (5'-GGTCGAGCTATACTGAG-TACGTCTT-3') and (5'-TCTTGCAGCAGCAGTCACA-3'); 60s: (5'-GGAAGTACCAGGCAGTGACAG -3') and (5'-GCAGGCATGAGGCAAACAG-3'). cDNA copy numbers for each gene were quantified by using standard curves of known quantities of plasmids containing the corresponding PCR products. 60S ribosomal protein L13A was used for normalization.



**Supplementary Figure 12****Loss of KCTD effects on activation and desensitization of hippocampal GABA<sub>B</sub> receptors upon preclusion of KCTD-GABA<sub>B2</sub> interaction.**

**a**, Representative currents through K<sub>i</sub>3 channels recorded in experiments as in Fig. 4d from cultured hippocampal neurons prepared from GABA<sub>B2</sub><sup>-/-</sup> mice and

transfected either with GABA<sub>B2</sub> WT or with a GABA<sub>B2</sub> mutant (GABA<sub>B2</sub>Δ811) that fully precludes binding of KCTD proteins in neurons and cultured cells. Current traces on the right panel are enlargements of those framed in the left panel; current and time scaling as indicated, agonist application given by horizontal bars. **b**, Bar graphs summarizing relative desensitization (left) and onset (right) of the K<sub>ir</sub>3 currents recorded in experiments as in (a) and in experiments where KCTD12 was co-transfected as indicated; data points are mean ± SD of 5-11 experiments. The values for both relative desensitization and 20-80% rise-time were significantly different for GABA<sub>B2</sub> WT and GABA<sub>B2</sub>Δ811 (P < 0.001, Dunnett's multiple comparison test). **c**, Left panel: SDS-PAGE separation of *anti-GABA<sub>B1</sub>* APs from CHO-cells expressing GABA<sub>B1</sub>, KCTD12 and either HA-tagged GABA<sub>B2</sub> or HA-tagged GABA<sub>B2</sub>Δ811; the gel separation was Western blotted with the indicated antibodies. Note the lack of interaction between KCTD12 and GABA<sub>B2</sub>(Δ811). Right panel: Bar graphs summarizing effects of KCTD12 on GABA<sub>B1</sub>/GABA<sub>B2</sub> or GABA<sub>B1</sub>/GABA<sub>B2</sub>Δ811 receptors heterologously expressed in CHO-cells and characterized in experiments as in Fig. 4a-c; data points are mean ± SD of 8-16 experiments. Inset: representative K<sub>ir</sub>3.1/3.2 currents recorded from CHO-cells expressing GABA<sub>B1</sub>/GABA<sub>B2</sub>Δ811 receptors in the absence (blue trace) and presence of KCTD12 (red trace).



### Supplementary Figure 13

#### Change in onset and desensitization of the GABA<sub>B</sub> response in hippocampal neurons upon knock-down of KCTD12 by shRNA.

**a**, Representative  $K_{ir3}$  currents recorded in experiments as in Fig. 4d from cultured hippocampal neurons transfected either with control shRNAs or with KCTD12-specific shRNA. Current traces on the right panel are enlargements of those framed in the left panel; current and time scaling as indicated, agonist application given by the horizontal bars. Solution exchange was approximated with a monoexponential function with a time constant of  $\sim 95$  ms. Note the significant slowing of activation and decrease in desensitization induced by knock-down of KCTD12. **b**, Bar graphs summarizing relative desensitization (left) and onset (right) of the  $K_{ir3}$  currents recorded in experiments as in (a); data are mean  $\pm$  SD of 9 (control shRNAs) and 10 (KCTD12 shRNA) experiments. The values for both relative desensitization and 20-80% rise-time were significantly different between control shRNAs and KCTD12-specific shRNA ( $P < 0.001$ , Dunnett's multiple comparison test).

Supplementary Table 1a

Accession	Protein name (Swiss-Prot)	<i>anti-GABA<sub>B1</sub></i> AP			<i>anti-GABA<sub>B2</sub></i> AP		
		log[ab <sub>norm</sub> ]	rPV <sub>IgG</sub>	rPV <sub>ko</sub>	log[ab <sub>norm</sub> ]	rPV <sub>IgG</sub>	rPV <sub>ko</sub>
<b>O88871</b>	<b>Gamma-aminobutyric acid type B receptor subunit 2</b>	<b>5.0</b>	1762	584	<b>5.6</b>	2490	3227
<b>Q920U4</b>	<b>Gamma-aminobutyric acid type B receptor subunit 1</b>	<b>5.1</b>	785	803	<b>5.5</b>	2048	2632
<b>Q6WVG3</b>	<b>BTB/POZ domain-containing protein KCTD12</b>	<b>5.1</b>	429	205	<b>5.5</b>	1018	1020
<b>Q5DTY9</b>	<b>BTB/POZ domain-containing protein KCTD16</b>	<b>5.0</b>	219	259	<b>5.4</b>	884	2489
P04797	Glyceraldehyde-3-phosphate dehydrogenase	4.8	19	3	5.0	15	9
<b>Q8C7J6</b>	<b>Putative uncharacterized protein = KCTD12b</b>	<b>4.7</b>	n.d.#	17	<b>5.0</b>	20	90
Q4QRB4	Tubulin beta-3 chain	5.0	54	3	4.7	51	18
<b>Q50H33</b>	<b>BTB/POZ domain-containing protein KCTD8</b>	<b>4.6</b>	112	119	<b>5.0</b>	288	944
<b>P69897</b>	<b>Tubulin beta-5 chain</b>	<b>4.8</b>	302	87	<b>4.8</b>	200	19
Q5XIF6	Tubulin alpha-4A chain	4.8	91	9	4.7	96	13
Q9D6F9	Tubulin beta-4 chain	4.7	22	2	4.7	87	45
P06687	Sodium/potassium-transporting ATPase subunit alpha-3	4.6	49	3	4.7	47	12
<b>P06685</b>	<b>Sodium/potassium-transporting ATPase subunit alpha-1</b>	<b>4.6</b>	119	44	<b>4.6</b>	74	51
<b>P06686</b>	<b>Sodium/potassium-transporting ATPase subunit alpha-2</b>	<b>4.6</b>	150	50	<b>4.6</b>	160	56
P07340	Sodium/potassium-transporting ATPase subunit beta-1	4.6	197	1	4.5	145	11
P12236	ADP/ATP translocase 3	4.5	188	n.d.	4.3	140	n.d.
P62944	AP-2 complex subunit beta-1	4.5	30	23	4.3	19	3
P62989	Ubiquitin	4.3	45	9	4.2	31	42
P13233	2',3'-cyclic-nucleotide 3'-phosphodiesterase	4.2	114	3	4.2	67	6
P47819	Glial fibrillary acidic protein *	4.3	10	3	4.0	19	2
P59215	Guanine nucleotide-binding protein G(o) subunit alpha	4.3	138	3	4.0	66	9
Q9QUL6	Vesicle-fusing ATPase	4.1	139	1	4.1	96	3
P63018	Heat shock cognate 71 kDa protein	4.1	12	2	4.0	10	3
P60203	Myelin proteolipid protein	4.1	62	3	4.0	77	6
P09606	Glutamine synthetase	4.1	78	n.d.	3.9	49	n.d.
P63012	Ras-related protein Rab-3A	4.1	48	3	3.9	23	4
<b>P11507</b>	<b>Sarcoplasmic/endoplasmic reticulum calcium ATPase 2</b>	<b>4.1</b>	242	106	<b>3.7</b>	108	45
P01830	Thy-1 membrane glycoprotein	4.1	109	2	3.7	68	60
Q9Z2L0	Voltage-dependent anion-selective channel protein 1	3.9	85	2	3.7	68	4
P54311	Guanine nucleotide-binding protein subunit beta-1	3.9	79	6	3.8	83	32
P31596	Excitatory amino acid transporter 2	4.0	102	22	3.6	57	7
P84092	AP-2 complex subunit mu-1	4.0	41	6	3.5	5	4
P05065	Fructose-bisphosphate aldolase A	3.7	83	n.d.	3.8	19	n.d.
P63102	14-3-3 protein zeta/delta	3.6	48	n.d.	3.8	46	40
P61265	Syntaxin-1B	3.6	57	3	3.7	30	67
P24942	Excitatory amino acid transporter 1	3.9	133	6	3.4	99	4
Q60931	Voltage-dependent anion-selective channel protein 3 *	3.7	27	2	3.6	8	24
Q62425	NADH dehydrogenase [ubiquinone] 1 alpha subunit 4 *	3.7	63	2	3.5	35	2
<b>P31647</b>	<b>Sodium- and chloride-dependent GABA transporter 3</b>	<b>3.6</b>	73	27	<b>3.6</b>	70	50
P47942	Dihydropyrimidinase-related protein 2 *	3.4	15	n.d.	3.8	69	1
P12273	Prolactin-inducible protein	3.6	34	n.d.	3.4	21	n.d.
Q15149	Plectin-1 **	3.5	33	n.d.	3.4	35	n.d.
P61765	Syntaxin-binding protein 1	3.5	54	2	3.4	14	49
Q4KM65	Cleavage and polyadenylation specificity factor subunit 5 **	3.6	24	2	3.3	30	1
<b>Q5PQL7</b>	<b>Integral membrane protein 2C **</b>	<b>2.9</b>	23	30	<b>4.0</b>	113	142
<b>P11506</b>	<b>Plasma membrane calcium-transporting ATPase 2</b>	<b>3.4</b>	38	34	<b>3.4</b>	21	33
P11505	Plasma membrane calcium-transporting ATPase 1	3.4	24	28	3.4	29	7
P63138	Gamma-aminobutyric acid receptor subunit beta-2	3.4	62	3	3.3	44	28
O43581	Synaptotagmin-7	3.7	65	1	2.8	22	17
P07335	Creatine kinase B-type	3.5	95	n.d.	3.0	37	n.d.
P13638	Sodium/potassium-transporting ATPase subunit beta-2 *	3.2	34	n.d.	3.3	65	n.d.
P62813	Gamma-aminobutyric acid receptor subunit alpha-1	3.2	81	8	3.1	50	50
Q5FWT1	Protein FAM98A	3.3	21	n.d.	3.1	14	n.d.
P63319	Protein kinase C gamma type **	3.2	14	n.d.	3.1	56	n.d.
Q9NVH2	Integrator complex subunit 7 *	3.2	54	n.d.	2.9	9	n.d.
P0C5X8	Protein tweety homolog 1	3.0	31	n.d.	3.0	34	n.d.
P54282	Voltage-dependent P/Q-type calcium channel sub. alpha-1A	3.2	24	n.d.	2.8	3	3
Q8CG48	Structural maintenance of chromosomes protein 2 *	2.8	9	n.d.	3.0	33	n.d.
P47860	6-phosphofructokinase type C	2.9	26	n.d.	2.9	26	n.d.



Supplementary Table 1b

Accession	Protein name (Swiss-Prot)	<i>anti-GABA<sub>B1</sub></i> AP		<i>anti-GABA<sub>B2</sub></i> AP	
		Mascot_Score	FDR <sub>decoy</sub>	Mascot_Score	FDR <sub>decoy</sub>
<b>O88871</b>	<b>Gamma-aminobutyric acid type B receptor subunit 2</b>	<b>1114</b>	<b>0</b>	<b>1454</b>	<b>0</b>
<b>Q920U4</b>	<b>Gamma-aminobutyric acid type B receptor subunit 1</b>	<b>1578</b>	<b>0</b>	<b>2117</b>	<b>0</b>
<b>Q6WVG3</b>	<b>BTB/POZ domain-containing protein KCTD12</b>	<b>563</b>	<b>0</b>	<b>756</b>	<b>0</b>
<b>Q5DTY9</b>	<b>BTB/POZ domain-containing protein KCTD16</b>	<b>523</b>	<b>0</b>	<b>819</b>	<b>0</b>
P04797	Glyceraldehyde-3-phosphate dehydrogenase	454	0	484	0
<b>Q8C7J6</b>	<b>Putative uncharacterized protein = KCTD12b</b>	<b>111</b>	<b>0</b>	<b>202</b>	<b>0</b>
Q4QRB4	Tubulin beta-3 chain	930	0	814	0
<b>Q50H33</b>	<b>BTB/POZ domain-containing protein KCTD8</b>	<b>331</b>	<b>0</b>	<b>485</b>	<b>0</b>
<b>P69897</b>	<b>Tubulin beta-5 chain</b>	<b>1042</b>	<b>0</b>	<b>962</b>	<b>0</b>
Q5XIF6	Tubulin alpha-4A chain	934	0	877	0
Q9D6F9	Tubulin beta-4 chain	1008	0	902	0
P06687	Sodium/potassium-transporting ATPase subunit alpha-3	1703	0	1594	0
<b>P06685</b>	<b>Sodium/potassium-transporting ATPase subunit alpha-1</b>	<b>1445</b>	<b>0</b>	<b>1231</b>	<b>0</b>
<b>P06686</b>	<b>Sodium/potassium-transporting ATPase subunit alpha-2</b>	<b>1525</b>	<b>0</b>	<b>1315</b>	<b>0</b>
P07340	Sodium/potassium-transporting ATPase subunit beta-1	330	0	316	0
P12236	ADP/ATP translocase 3	373	0	401	0
P62944	AP-2 complex subunit beta-1	<b>372</b>	<b>0</b>	<b>195</b>	<b>0</b>
P62989	Ubiquitin	187	0	<b>203</b>	<b>0</b>
P13233	2',3'-cyclic-nucleotide 3'-phosphodiesterase	553	0	197	0
P47819	Glial fibrillary acidic protein	77	0.01	146	0
P59215	Guanine nucleotide-binding protein G(o) subunit alpha	336	0	268	0
Q9QUL6	Vesicle-fusing ATPase	750	0	782	0
P63018	Heat shock cognate 71 kDa protein	605	0	568	0
P60203	Myelin proteolipid protein	182	0	n.d.	n.d.
P09606	Glutamine synthetase	222	0	152	0
P63012	Ras-related protein Rab-3A	215	0	185	0
<b>P11507</b>	<b>Sarcoplasmic/endoplasmic reticulum calcium ATPase 2</b>	<b>940</b>	<b>0</b>	<b>338</b>	<b>0</b>
P01830	Thy-1 membrane glycoprotein	133	0	n.d.	n.d.
Q9Z2L0	Voltage-dependent anion-selective channel protein 1	277	0	238	0
P54311	Guanine nucleotide-binding protein subunit beta-1	156	0	<b>142</b>	<b>0</b>
P31596	Excitatory amino acid transporter 2	<b>269</b>	<b>0</b>	188	0
P84092	AP-2 complex subunit mu-1	292	0	112	0
P05065	Fructose-bisphosphate aldolase A	246	0	66	0
P63102	14-3-3 protein zeta/delta	120	0	<b>228</b>	<b>0</b>
P61265	Syntaxin-1B	n.d.	n.d.	<b>135</b>	<b>0</b>
P24942	Excitatory amino acid transporter 1	160	0	60	0
Q60931	Voltage-dependent anion-selective channel protein 3	68	0.02	67	0
Q62425	NADH dehydrogenase [ubiquinone] 1 alpha subunit 4	106	0	48	0.04
<b>P31647</b>	<b>Sodium- and chloride-dependent GABA transporter 3</b>	<b>128</b>	<b>0</b>	<b>161</b>	<b>0</b>
P47942	Dihydropyrimidinase-related protein 2	58	0.03	467	0
P12273	Prolactin-inducible protein	196	0	59	0
Q15149	Plectin-1	49	0.07	n.d.	n.d.
P61765	Syntaxin-binding protein 1	171	0	336	0
Q4KM65	Cleavage and polyadenylation specificity factor subunit 5	36	0.15	96	0
<b>Q5PQL7</b>	<b>Integral membrane protein 2C</b>	<b>43</b>	<b>0.09</b>	<b>275</b>	<b>0</b>
<b>P11506</b>	<b>Plasma membrane calcium-transporting ATPase 2</b>	<b>434</b>	<b>0</b>	<b>428</b>	<b>0</b>
P11505	Plasma membrane calcium-transporting ATPase 1	<b>411</b>	<b>0</b>	n.d.	n.d.
P63138	Gamma-aminobutyric acid receptor subunit beta-2	104	0	<b>85</b>	<b>0</b>
O43581	Synaptotagmin-7	241	1	<b>n.d.</b>	<b>n.d.</b>
P07335	Creatine kinase B-type	143	0	n.d.	n.d.
P13638	Sodium/potassium-transporting ATPase subunit beta-2	67	0.02	<b>66</b>	<b>0</b>
P62813	Gamma-aminobutyric acid receptor subunit alpha-1	95	0	<b>92</b>	<b>0</b>
Q5FWT1	Protein FAM98A	226	0	n.d.	n.d.
P63319	Protein kinase C gamma type	n.d.	n.d.	<b>39</b>	0.13
Q9NVH2	Integrator complex subunit 7	51	0.05	n.d.	n.d.
P0C5X8	Protein tweety homolog 1	105	0	116	0
P54282	Voltage-dependent P/Q-type calcium channel sub. alpha-1A	n.d.	n.d.	<b>58</b>	<b>0</b>
Q8CG48	Structural maintenance of chromosomes protein 2	60	0.02	<b>54</b>	<b>0</b>
P47860	6-phosphofructokinase type C	136	0	124	0

**Supplementary Table 1****List of proteins identified in APs from rat brain with both *anti-GABA<sub>B1</sub>* and *anti-GABA<sub>B2</sub>* antibodies (see Fig. 1b)**

**a**, Parameters for each protein (referenced to the Swiss-Prot database) are (from left to right): normalized protein abundance (given as  $\log[ab_{norm}]$ ; see Fig. 1b), peak volume relative to IgG control ( $rPV_{IgG}$ , see Methods), peak volume relative to knock-out control ( $rPV_{ko}$ , see Methods).  $rPV$  values compare the amount of each protein identified in APs with the *anti-GABA<sub>B</sub>* antibodies from rat and mouse WT brains with the respective amount determined in APs either with IgGs (rat,  $rPV_{IgG}$ ) or with *anti-GABA<sub>B</sub>* from GABA<sub>B</sub> core subunit knock-out animals ( $rPV_{ko}$ ).  $rPV$  values are calculated as the median of the ratios of the six most consistent tryptic peptides of each protein (if the MS signal of a given peptide was not detected in the controls, the detection threshold of the spectrometer (3000 volume units at the settings used) served as the denominator in  $rPV$  calculations). Threshold values of 20 for ( $rPV_{IgG}$ ) and 10 (for  $rPV_{ko}$ ) were used to define specific co-purification of candidate proteins with the indicated antibody (bold values highlighted in yellow). n.d. (not determined) denotes failure of MS analysis to provide definitive information for a given protein. Note that only a few proteins were abundantly and specifically co-purified with both *anti-GABA<sub>B</sub>* antibodies (proteins highlighted in yellow, corresponding to the filled circles in Fig. 1b). Asterisks denote proteins identified with a false discovery probability ( $FDR_{decoy}$ ) of  $0 < FDR < 0.05$  (\*) or  $0.05 < FDR < 0.15$  (\*\*).

**b**, Protein list as in a indicating the cumulative MASCOT score and  $FDR_{decoy}$  values.  $FDR_{decoy}$  was calculated as follows:

$$FDR = \text{Sum of false-positive hits (FH)} / \text{Sum of all hits (AH)}$$

for a protein with a Mascot score of  $i$ :

$$FH = (\text{Sum of decoy database hits with Mascot scores of } \geq i) \cdot 2$$

$$AH = \text{Sum of target-decoy database hits with Mascot scores of } \geq i$$

Peptides / Spectra obtained for GABA<sub>B</sub> core and KCTD proteins

Protein	Sequence	Mass	Modifications	Position	Charge	MissCLV	anti-GABA <sub>B</sub> (rat wt)			anti-KCTD12 (rat wt)			anti-KCTD16 (rat wt)							
							Count	Score	MS/MS Time	Exp Mass	Δm (ppm)	Count	Score	MS/MS Time	Exp Mass	Δm (ppm)	Count	Score	MS/MS Time	Exp Mass
GABR1_RAT Q9Z0J4	TLPEPK	772.4119			2	2	21	1841	772.412	0.1295	1	21	1840	772.412	0.1295	2	25	1830	772.412	0.1295
	PTFFR	813.4174			2	2	29	2501	813.4174	-0.2459	6	23	2467	813.4174	0.0000	2	23	2460	813.4178	0.4818
	DLVSWK	849.5988			2	2	35	1767	849.5988	-0.4709	2	40	1779	849.5988	-0.2300	0	43	1692	843.4476	0.1380
	WCEVYK	943.4476	Carbamidomethyl (C)	0.0100000.0	2	2	29	1655	943.4482	0.6339	1	25	1703	943.4480	0.7420	2	34	1688	943.4468	-0.3300
	SVYLENGK	1023.5237	Deamidated (NQ)	0.000002000.0	2	2	25	1849	1023.5238	0.0931	2	45	1854	1023.5244	0.6839	2	44	1854	1023.5206	-0.0977
	KWFEVYK	1074.5077	Carbamidomethyl (C)	0.0010000.0	2	1	47	1529	1074.5078	0.0977	1	53	1842	1074.5084	0.4890	2	45	1927	1074.5244	-4.1971
	WCEVYK	1089.5595	Glu-pyro-Glu (N-term O)	3.0000000.0	2	1	4	26	1089.5594	0.2800	1	33	1504	1071.5426	0.2800	2	38	1486	1071.5426	0.2800
	ORPFFER	1097.5771			2	1	4	26	1097.5770	0.3239	1	35	2015	1097.5774	0.2733	2	35	2235	1097.5769	0.8200
	DLVYELK	1104.5703	Carbamidomethyl (C)	0.0100000.0	2	1	2	35	1104.5708	0.4537	1	28	2408	1104.5710	0.6337	4	29	2422	1104.5694	-0.8148
	WCEVYK	1238.5600	Carbamidomethyl (C)	0.0000000.0	2	1	2	35	1238.5606	0.6884	1	23	1566	1238.5602	0.9767	0	54	2280	1238.5678	0.9688
	SQFFPK	1238.5686	Carbamidomethyl (C)	0.0000000.0	2	1	2	35	1238.5694	0.8455	0	35	2242	1238.5684	1.4633	0	54	2280	1238.5678	0.9688
	ROLDFLEK	1260.6714			3	1	2	29	1260.6721	0.5453	0	38	2165	1260.6744	2.3797	2	38	2150	1260.6724	0.7932
	ROLDFLEK	1260.6714			3	1	2	29	1260.6714	1.5885	1	32	2143	1260.6734	-0.0933	2	29	2143	1260.6732	0.1586
	WVAGIEFR	1261.7000			3	1	2	29	1261.7029	-0.0933	1	32	2143	1261.7029	-0.0933	2	29	2143	1261.7032	0.1586
	WVAGIEFR	1261.7000			3	1	2	29	1261.7000	0.4838	2	27	2686	1303.6446	-0.2001	2	53	2675	1303.6462	0.9972
	WVAGIEFR	1261.7000			3	1	2	29	1261.7000	0.4838	2	27	2686	1303.6446	-0.2001	2	53	2675	1303.6462	0.9972
	WVAGIEFR	1261.7000			3	1	2	29	1261.7000	0.4838	2	27	2686	1303.6446	-0.2001	2	53	2675	1303.6462	0.9972
	WVAGIEFR	1261.7000			3	1	2	29	1261.7000	0.4838	2	27	2686	1303.6446	-0.2001	2	53	2675	1303.6462	0.9972
	WVAGIEFR	1261.7000			3	1	2	29	1261.7000	0.4838	2	27	2686	1303.6446	-0.2001	2	53	2675	1303.6462	0.9972
	WVAGIEFR	1261.7000			3	1	2	29	1261.7000	0.4838	2	27	2686	1303.6446	-0.2001	2	53	2675	1303.6462	0.9972
	WVAGIEFR	1261.7000			3	1	2	29	1261.7000	0.4838	2	27	2686	1303.6446	-0.2001	2	53	2675	1303.6462	0.9972
	WVAGIEFR	1261.7000			3	1	2	29	1261.7000	0.4838	2	27	2686	1303.6446	-0.2001	2	53	2675	1303.6462	0.9972
	WVAGIEFR	1261.7000			3	1	2	29	1261.7000	0.4838	2	27	2686	1303.6446	-0.2001	2	53	2675	1303.6462	0.9972
	WVAGIEFR	1261.7000			3	1	2	29	1261.7000	0.4838	2	27	2686	1303.6446	-0.2001	2	53	2675	1303.6462	0.9972
	WVAGIEFR	1261.7000			3	1	2	29	1261.7000	0.4838	2	27	2686	1303.6446	-0.2001	2	53	2675	1303.6462	0.9972
	WVAGIEFR	1261.7000			3	1	2	29	1261.7000	0.4838	2	27	2686	1303.6446	-0.2001	2	53	2675	1303.6462	0.9972
	WVAGIEFR	1261.7000			3	1	2	29	1261.7000	0.4838	2	27	2686	1303.6446	-0.2001	2	53	2675	1303.6462	0.9972
	WVAGIEFR	1261.7000			3	1	2	29	1261.7000	0.4838	2	27	2686	1303.6446	-0.2001	2	53	2675	1303.6462	0.9972
	WVAGIEFR	1261.7000			3	1	2	29	1261.7000	0.4838	2	27	2686	1303.6446	-0.2001	2	53	2675	1303.6462	0.9972
	WVAGIEFR	1261.7000			3	1	2	29	1261.7000	0.4838	2	27	2686	1303.6446	-0.2001	2	53	2675	1303.6462	0.9972
	WVAGIEFR	1261.7000			3	1	2	29	1261.7000	0.4838	2	27	2686	1303.6446	-0.2001	2	53	2675	1303.6462	0.9972
	WVAGIEFR	1261.7000			3	1	2	29	1261.7000	0.4838	2	27	2686	1303.6446	-0.2001	2	53	2675	1303.6462	0.9972
	WVAGIEFR	1261.7000			3	1	2	29	1261.7000	0.4838	2	27	2686	1303.6446	-0.2001	2	53	2675	1303.6462	0.9972
WVAGIEFR	1261.7000			3	1	2	29	1261.7000	0.4838	2	27	2686	1303.6446	-0.2001	2	53	2675	1303.6462	0.9972	
WVAGIEFR	1261.7000			3	1	2	29	1261.7000	0.4838	2	27	2686	1303.6446	-0.2001	2	53	2675	1303.6462	0.9972	
WVAGIEFR	1261.7000			3	1	2	29	1261.7000	0.4838	2	27	2686	1303.6446	-0.2001	2	53	2675	1303.6462	0.9972	
WVAGIEFR	1261.7000			3	1	2	29	1261.7000	0.4838	2	27	2686	1303.6446	-0.2001	2	53	2675	1303.6462	0.9972	
WVAGIEFR	1261.7000			3	1	2	29	1261.7000	0.4838	2	27	2686	1303.6446	-0.2001	2	53	2675	1303.6462	0.9972	
WVAGIEFR	1261.7000			3	1	2	29	1261.7000	0.4838	2	27	2686	1303.6446	-0.2001	2	53	2675	1303.6462	0.9972	
WVAGIEFR	1261.7000			3	1	2	29	1261.7000	0.4838	2	27	2686	1303.6446	-0.2001	2	53	2675	1303.6462	0.9972	
WVAGIEFR	1261.7000			3	1	2	29	1261.7000	0.4838	2	27	2686	1303.6446	-0.2001	2	53	2675	1303.6462	0.9972	
WVAGIEFR	1261.7000			3	1	2	29	1261.7000	0.4838	2	27	2686	1303.6446	-0.2001	2	53	2675	1303.6462	0.9972	
WVAGIEFR	1261.7000			3	1	2	29	1261.7000	0.4838	2	27	2686	1303.6446	-0.2001	2	53	2675	1303.6462	0.9972	
WVAGIEFR	1261.7000			3	1	2	29	1261.7000	0.4838	2	27	2686	1303.6446	-0.2001	2	53	2675	1303.6462	0.9972	
WVAGIEFR	1261.7000			3	1	2	29	1261.7000	0.4838	2	27	2686	1303.6446	-0.2001	2	53	2675	1303.6462	0.9972	
WVAGIEFR	1261.7000			3	1	2	29	1261.7000	0.4838	2	27	2686	1303.6446	-0.2001	2	53	2675	1303.6462	0.9972	
WVAGIEFR	1261.7000			3	1	2	29	1261.7000	0.4838	2	27	2686	1303.6446	-0.2001	2	53	2675	1303.6462	0.9972	
WVAGIEFR	1261.7000			3	1	2	29	1261.7000	0.4838	2	27	2686	1303.6446	-0.2001	2	53	2675	1303.6462	0.9972	
WVAGIEFR	1261.7000			3	1	2	29	1261.7000	0.4838	2	27	2686	1303.6446	-0.2001	2	53	2675	1303.6462	0.9972	
WVAGIEFR	1261.7000			3	1	2	29	1261.7000	0.4838	2	27	2686	1303.6446	-0.2001	2	53	2675	1303.6462	0.9972	
WVAGIEFR	1261.7000			3	1	2	29	1261.7000	0.4838	2	27	2686	1303.6446	-0.2001	2	53	2675	1303.6462	0.9972	
WVAGIEFR	1261.7000			3	1	2	29	1261.7000	0.4838	2	27	2686	1303.6446	-0.2001	2	53	2675	1303.6462	0.9972	
WVAGIEFR	1261.7000			3	1	2	29	1261.7000	0.4838	2	27	2686	1303.6446	-0.2001	2	53	2675	1303.6462	0.9972	
WVAGIEFR	1261.7000			3	1	2	29	1261.7000	0.4838	2	27	2686	1303.6446	-0.2001	2	53	2675	1303.6462	0.9972	
WVAGIEFR	1261.7000			3	1	2	29	1261.7000	0.4838	2	27	2686	1303.6446	-0.2001	2	53	2675	1303.6462	0.9972	
WVAGIEFR	1261.7000			3	1	2	29	1261.7000	0.4838	2	27	2686	1303.6446	-0.2001	2	53	2675	1303.6462	0.9972	
WVAGIEFR	1261.7000			3	1	2	29	1261.7000	0.4838	2	27	2686	1303.6446	-0.2001	2	53	2675	1303.6462	0.9972	
WVAGIEFR	1261.7000			3	1	2	29	1261.7000	0.4838	2	27	2686	1303.6446	-0.2001	2	53	2675	1303.6462</		









**Supplementary Table 2****List of MS/MS spectra assigned to GABA<sub>B1</sub>, GABA<sub>B2</sub>, KCTD8, KCTD12, KCTD12b and KCTD16 (see Supplementary Fig. 4)**

Mascot results of nano-LC MS/MS analyses of the indicated APs searched in the Swiss-Prot database. Data listed are (from left to right): target protein (Swiss-Prot entry and database accession), primary sequence, and mass of the respective peptide, peptide modification(s), position of the modification within the primary sequence of the peptide, charge of the peptide, number of miscleavages. These data are followed by number of assigned spectra (count), Mascot score (score), and time point of acquisition of the respective MS/MS spectrum (MS2 time), measured mass (ExpMass) and mass error in ppm for each peptide. Peptides specific for the respective target protein are in black, shared peptides are in grey.