Supporting Information

Pétriz et al. 10.1073/pnas.1419632111

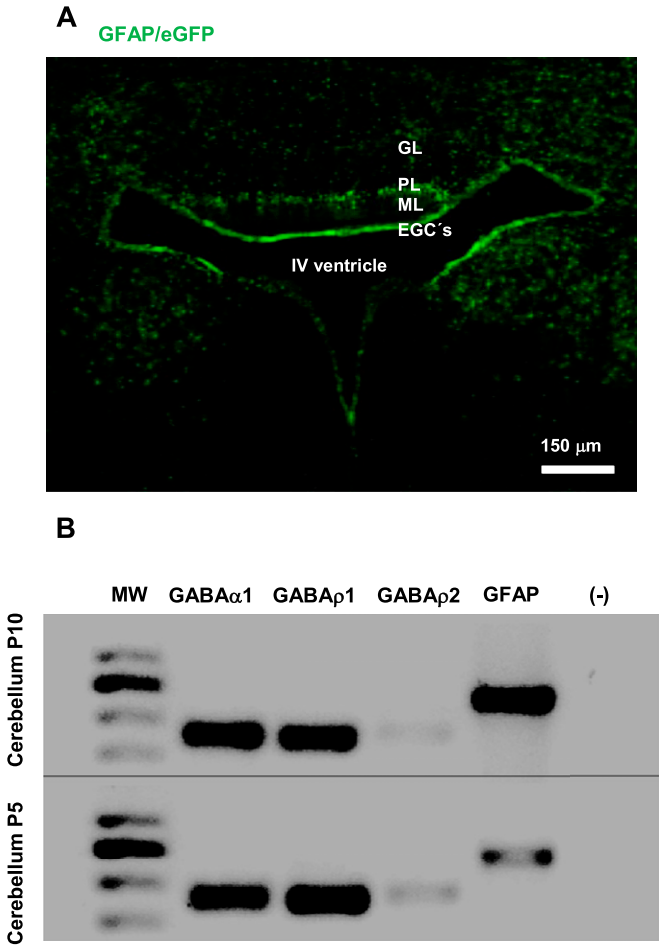

Fig. S1. (*A*) Coronal slice (40 μm thick) of cerebellum showing the location of EGCs that face the fourth ventricle and the distribution of cerebellar GFAP$^+$ cells in this area. PL, Purkinje layer; ML, molecular layer; 4V, fourth ventricle. (*B*) mRNA expression of GABAα1, GABAγ2, GABAρ1, GABAρ2, and GFAP in cerebellum from P5 and P10 mice.

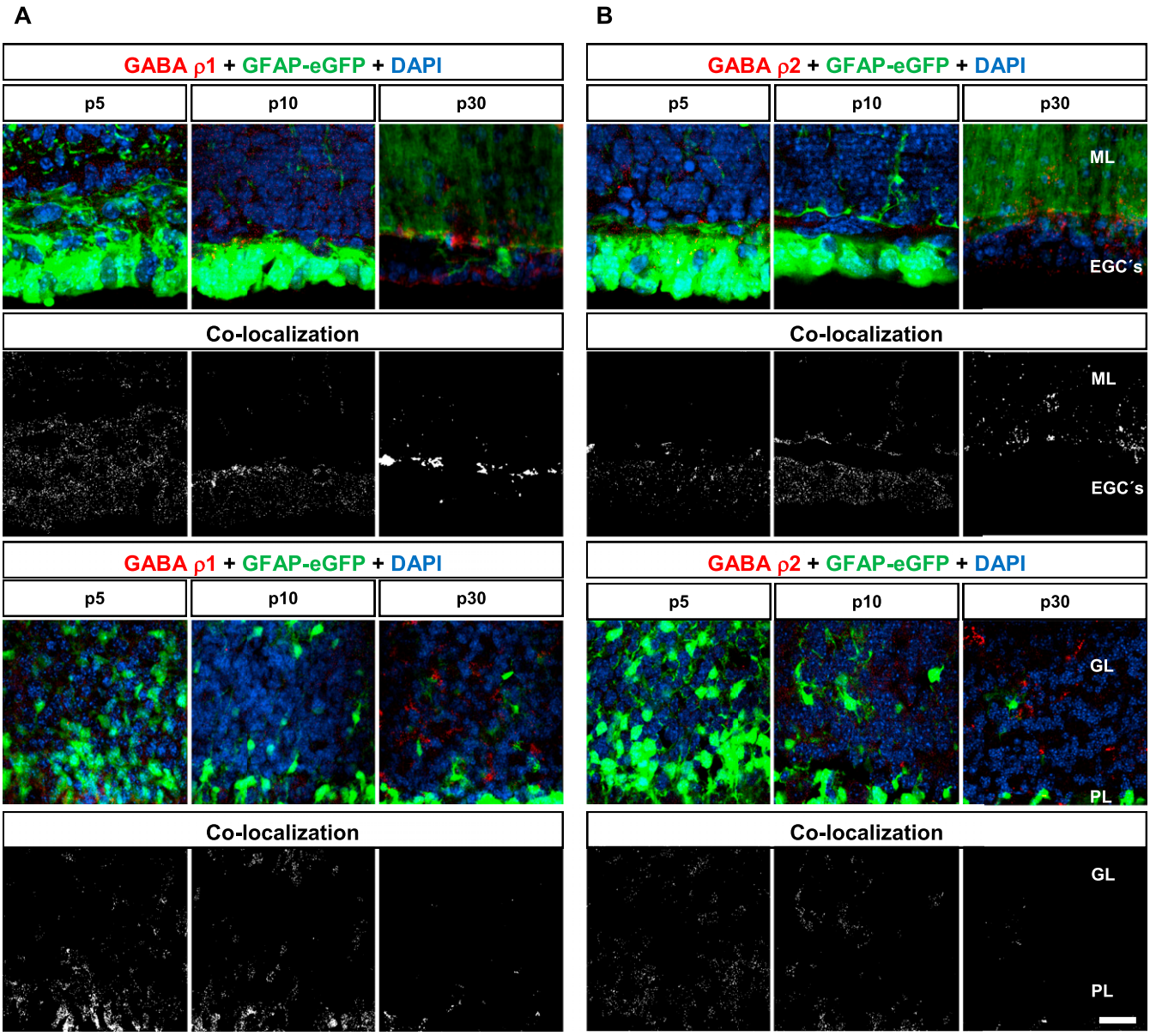

Fig. S2. (*A*) Localization of GABAρ1 (red) in GFAP$^+$ cells (green) in P5, P10, and P30 mice. White dots indicate the colocalization pattern over time in EGCs and in GFAP cells of the GL. (*B*) Colocalization of GABAρ2 (red) and GFAP$^+$ cells (green) in P5, P10, and P30 mice is higher at P5 than at P10 or P30. ML, molecular layer; PL, Purkinje layer. (Scale bar: 50 μm.)

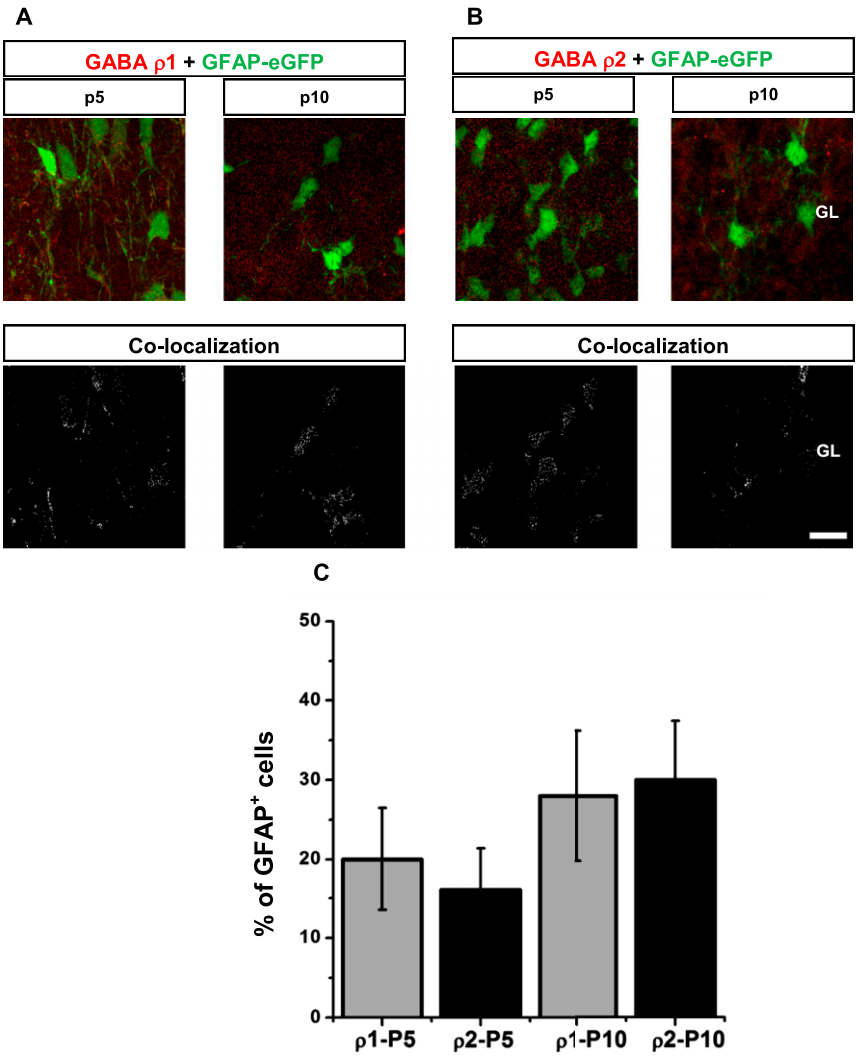

Fig. S3. (*A* and *B*) Colabeling of GABAρ1 or GABAρ2 with GFAP$^+$ cells from the GL of cerebellum at P5 and P10. (*C*) Percentage of GFAP$^+$ cells from the GL that express GABAρ1 and GABAρ2 at P5 and P10. $n = 3$. (Scale bar: 20 μm.)

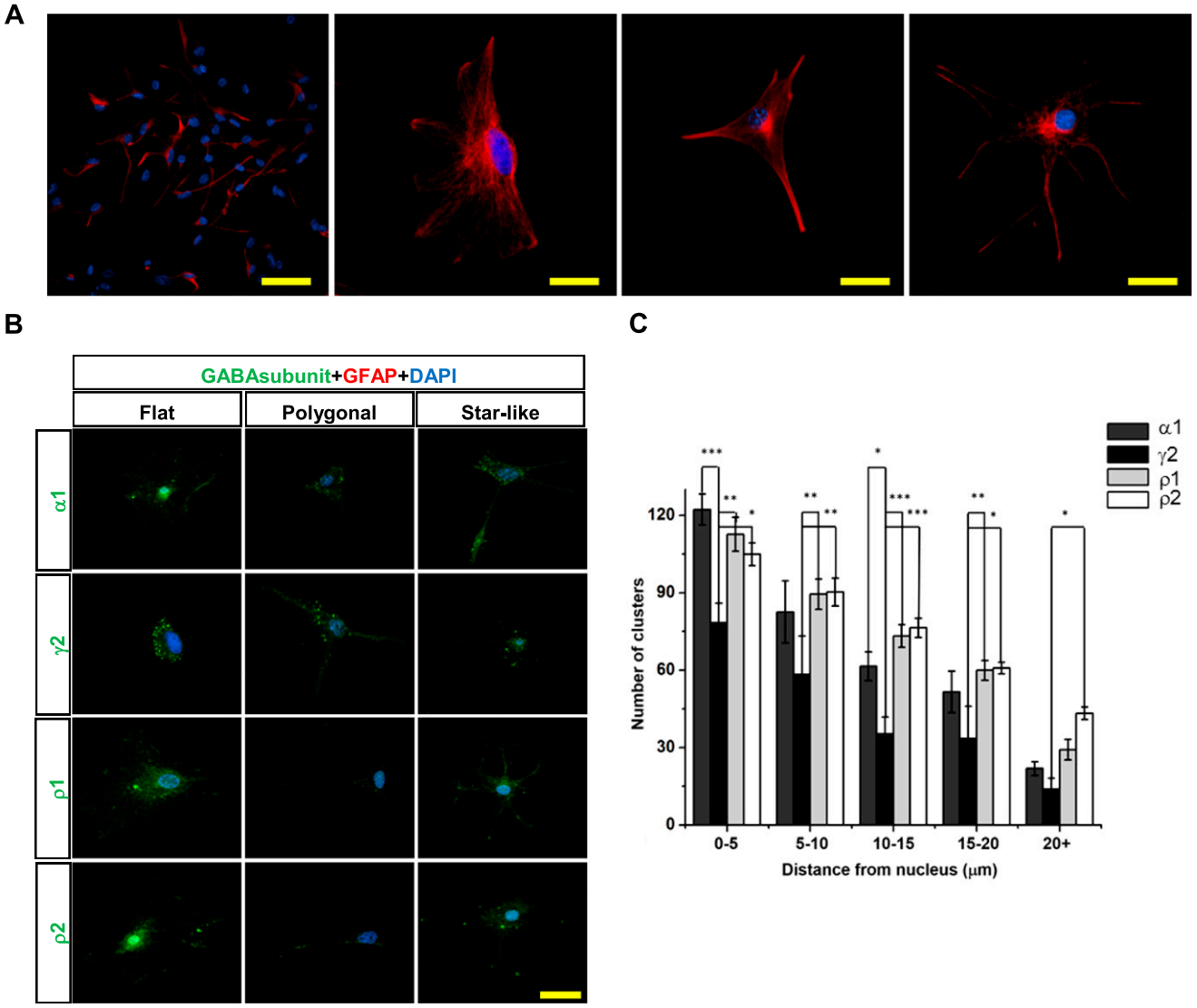

Fig. S4. (*A*) Morphologies of GFAP$^+$ cells in primary culture. From left to right, panoramic view of cerebellar cells in culture and examples of flat, polygonal, and star-like cells. (*B*) Distribution of GABAα1, GABAγ2, GABAρ1, and GABAρ2 in GFAP$^+$ cells in culture. Nuclei were stained with DAPI (blue). (Scale bars: 50 and 20 μm.) (*C*) Distribution of GABA subunit fluorescent clusters. GABAγ2 was significantly different from that of the other subunits. $n = 18$ cells. ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$.

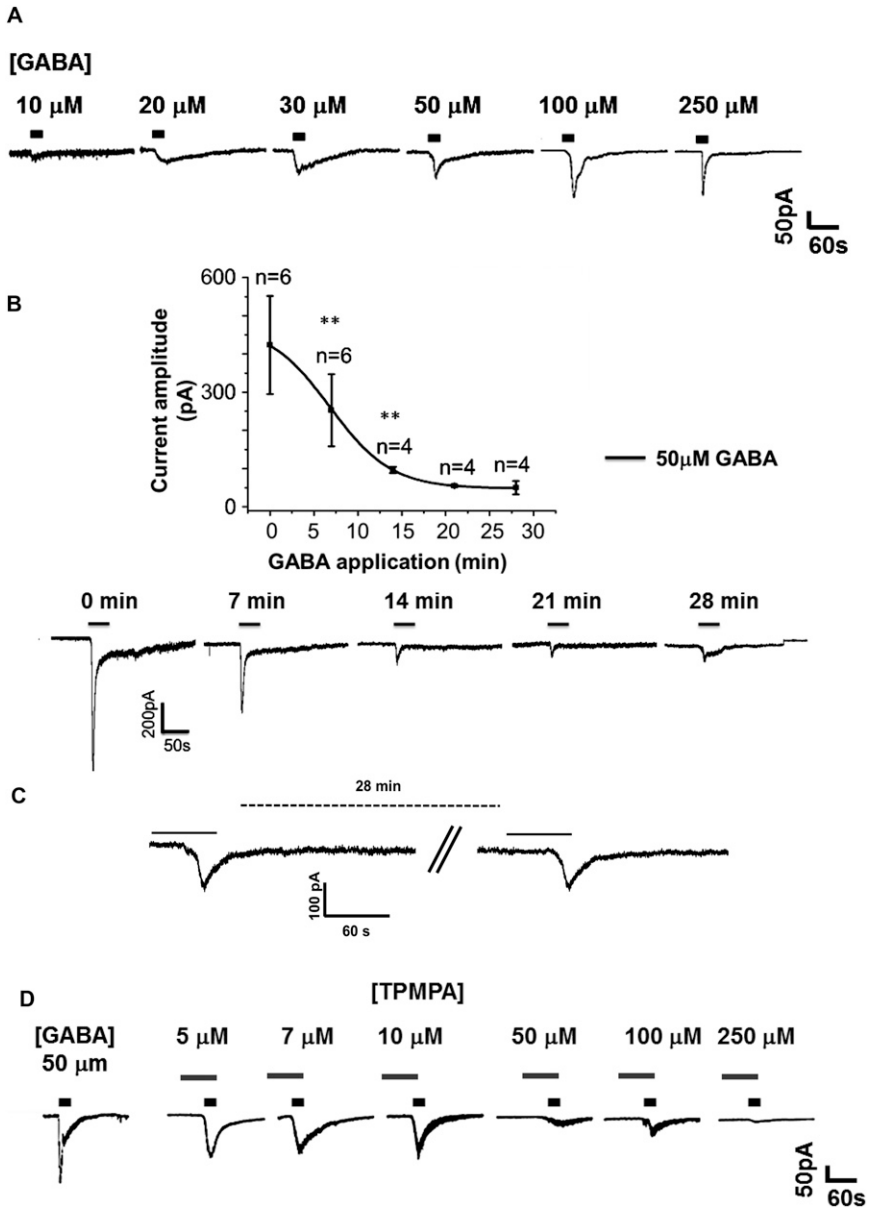

Fig. S5. (*A*) Sample recordings of GABA responses from astrocytes in culture. (*B*) GABA-desensitization curve after repeated applications of 50 μM GABA. Note the nondesensitizing component remaining after 28 min. (*C*) GABA responses after two applications of GABA 50 μM and 28 min of washing perfusion. (*D*) Sample recordings of GABA responses blocked by increasing concentrations of TPMPA. $n = 6$ cells for application.

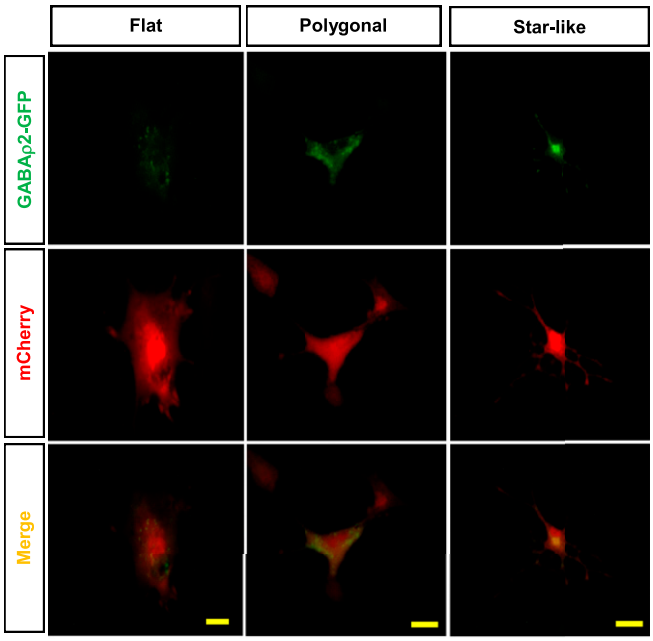

Fig. S6. Fluorescence emitted by GABAρ2–GFP (green) and soluble mCherry (red) in flat, polygonal, and star-like cells in culture. (Scale bar: 25 μm.)

Table S1. Antibodies for immunofluorescence, immunogold, and Western blot analyses

Antibody	Company	Catalog no.	Use
Primary antibodies			
Rabbit IgG anti-GABAα1	Millipore	06–868	WB, IF
Rabbit IgG anti-GABAα1–6	Santa Cruz Biotechnology	sc-14005	WB, IF
Rabbit IgG anti-GABAρ1	Santa Cruz Biotechnology	sc-25707	WB, IF, IG
Goat IgG anti-GABAρ1	Santa Cruz Biotechnology	sc-21338	WB, IF
Goat IgG anti-GABAρ2	Santa Cruz Biotechnology	sc-30254	WB, IF
Goat IgG anti-GFAP	Santa Cruz Biotechnology	sc-6171	WB, IF
Guinea pig anti-GABAγ2	Synaptic Systems	224004	IF
Secondary antibodies			
Goat anti-rabbit IgG EM-grade 25 nm	Electron Microscopy Sciences	25116	IG
Rabbit anti-goat IgG-AP	Santa Cruz Biotechnology	sc-2771	WB
Goat anti-rabbit IgG-AP	Santa Cruz Biotechnology	sc-2034	WB
Alexa Fluor 594-conjugated donkey anti-goat	Molecular Probes	A-11058	IF
Alexa Fluor 594-conjugated donkey anti-rabbit	Molecular Probes	R37119	IF
Alexa Fluor 488-conjugated donkey anti-rabbit	Molecular Probes	R37118	IF

IF, immunofluorescence; IG, immunogold; WB, Western blot.

Table S2. Primers for RT-PCR GABA-A subunits and GFAP detection

	Primer		
Gene	Forward	Reverse	Product, pb
GABAα1	5′-tatggacagccctcccaagatgaac-3′	5′-catatcgtggtctgaaactggtccg-3′	177
GABAγ2	5′-tgcccaaacctggtatgacagacg-3′	5′-taactggagaactccaggggcagg-3′	289
GABAρ1	5′-cgaggagcacacgacgatgcc-3′	5′-ctgcacatccacgcccacagg-3′	197
GABAρ2	5′-cctgatggctctcgtggagag-3′	5′-ccaaaggctggcctcatggtg-3′	197
GFAP	5′-agccagcagaggcagggcagg-3′	5′-tctctgcacgctcgctcgccc-3′	239