Supplementary Material

Reverse dot Blot

Positively charged nylon membranes (Roche) were cut into strips. 50 ng of GluA1, GluA2, β3-Tub, GluA2 Flip, GluA2 Flop sense riboprobes (without DIG incorporation) were spotted onto the strips. The spots were air dried and then UV cross-linked to strips for 15 min. Strips were washed twice with washing buffer 1x(Maleic Acid 10mM;NaCl 15 mM, pH:7), pre-hybridized at 45°C for 2h and then hybridized at 62°C O/N with DIG-labeled antisense probes in different experiments. After post-hybridization washes, detection was done using an anti-DIG–alkaline phosphatase conjugate antibody and the incubation with CDP-star (Disodium 4-chloro-3-(methoxyspiro {1,2-dioxetane-3,2'-(5'-chloro) tricyclo [3.3.1.1^{3,7}]decan}-1-4-yl) phenyl phosphate). Membranes were exposed to X-ray film and chemiluminescent signal detected. A spot signal is visualized only when antisense probes bind to the complimentary spotted sense probes assessing the antisense riboprobes specificity.

PC12 cell culture and transfection

PC12 cells were grown in RPMI-1640 (Invitrogen) medium, containing penicillin and streptomycin supplemented with 2mM glutamine, 5% foetal calf serum and 10% horse serum at 37°C in a 5% CO_2 atmosphere using standard cell culture methods. Cells were plated at a density of 30000 cells/cm^2 and transfected using paramagnetic nanobeads (NeuroMag, Oz Biosciences, Marseille, France) as described in the main text with pEGFP-c1 base vector expressing GluA2 FlipA or GluA2 Flip G and GluA2 Flop.

Total RNA from transfected cells was extracted using a Qiagen Micro Kit according to the manufacturer's instructions (Qiagen, Milan, Italy). RNA quantification and quality controls were spectrophotometrically determined using AGILENT Bioanalyzer 2100 lab-on-a-chip technology (AGILENT Technologies, Santa Clara, CA, USA).

In situ and Padlock probe Hybridization on PC12 cells

Fluorescence *in situ* hybridization and Padlock probe hybridization on PC12 cells were performed as described in the main text.

RNA expression analysis by Agilent 2100 Bioanalyzer

We used the Agilent 2100 Bioanalyzer (Agilent Technologies, Waldbronn, Germany) to determine transcription efficiency of the GluA2-FlipA, GluA2-FlipG, MAP2-DTE vectors.

Co-amplification reactions were performed using primer pairs detecting EGFP mRNA to test vectors transcription efficiency and primers detecting β-Actin mRNA used as normalization control (Table S3). The Bioanalyzer uses lab-on-a-chip technology to perform gel electrophoresis of PCR products. Samples are separated electrophoretically in a polymer solution, similar to capillary electrophoresis. The instrument detects laser-induced fluorescence using an intercalating dye, which is added to the polymer. The bioanalyzer software automatically calculates the size and concentration of each separate band and displays the result in real time generating an electropherogram. The PCR products were analysed using DNA 1000 Lab chips kit (Agilent Technologies, Waldbronn, Germany). All the chips were prepared according to the manufacturer's instructions. In brief, the gel matrix was prepared by adding 25 μl of dye to a gel vial. The gel/dye mixture was passed through a spin filter. Each chip was filled with 9 μl of the gel/dye mixture. Samples and ladder wells were filled with 5 μl of DNA marker solution before adding 1 μl of ladder and samples (total 50-500 ng) in the respective wells. The chips were vortexed and placed in the bioanalyzer. The ratio of the concentration of each amplified product to that of the co-amplified housekeeping gene allowed a relative estimate of the mRNA level. (1-2)

Western Blot

Cells were solubilised with modified RIPA (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% NP-40, 0.25% NaDOC, 0.1% SDS and 1X protease inhibitor tablets; Roche Applied Science) and then sonicated. A portion of the lysate was used for the BCA protein concentration assay (Sigma-Aldrich). Before electrophoresis, each sample was incubated at 75°C for 10 minutes with reducing agent (Invitrogen). Equal amounts of protein were applied to precast SDS polyacrylamide gels (4–12% NuPAGE Bis-Tris gels; Invitrogen) and the proteins were electrophoretically transferred to a nitrocellulose Transfer Membrane (GE Healthcare, Waukesha, WI, USA). The membranes were blocked for 60 min with 5% non-fat dry milk in TBS-T (Tris-buffered saline with 0.1% Tween-20, Sigma-Aldrich) and then incubated overnight at 4°C in the blocking solution with both primary antibodies simultaneously (mouse monoclonal anti-GAPDH (1:40000, Sigma-Aldrich) and rabbit polyclonal anti-GFP (1:1000; Invitrogen)). Subsequently, the membranes were incubated for one hour at RT, with both IR-labeled secondary antibodies simultaneously (IR Dye680LT goat anti-mouse and IR Dye800CW goat anti-rabbit M-Medical Milan). Western blots were imaged with the IR Imaged (ODYSSEY LI-COR) in both 700 and 800 nm channels in a single scan. For each investigated protein, the intensity of the immunoreactive bands was analyzed with Image Studio 1.1 software. Data are presented as the ratio of the optical density of the investigated EGFP bands and the GAPDH bands.

Table S1: Primer sequences for In Vitro Transcription

rG1-T7-F	GAAATTAATACGACTCACTATAGGGAGACCTGCAGCAGGTGCGCTTCGAA
rG1-Sp6 –R	ACGATTTAGGTGACACTATAGAAGCACGTCTGCTCTTCCATAGACCAGT
rG2-T7-F	GAAATTAATACGACTCACTATAGGGAGAGTTTCGCAGTCACCAATGCTTTCTGCTCC
rG2-Sp6 –R	ACGATTTAGGTGACACTATAGAAGCATGTTTTCCAATGGTAATAACCTGGTCC
rG2-Flip-T7-F	GAAATTAATACGACTCACTATAGGGAGAAACCCCAGTAAATCTTGCAGT
rG2-Flip-Sp6–R	ACGATTTAGGTGACACTATAGAAGCTTACTTCCCGAGTCCTTGG
rG2-Flop-T7-F	GAAATTAATACGACTCACTATAGGGAGAAAATGCGGTTAACCTCGCAG
rG2-Flop-Sp6–R	ACGATTTAGGTGACACTATAGAAGCTTGGAATCACCTCCCCC
rTubb3–T7–F	GAAATTAATACGACTCACTATAGGGAGAAGCCTGGAACCATGGACAGCG
rTubb3–sp6–R	ACGATTTAGGTGACACTATAGAAGGACACAAGGTGGTTGAGGTCCC
EGFP-Sp6-F	ATTTAGGTGACACTATAGAAGGACCACATGAAGCAGCACGACTTCTT
EGFP-T7-R	TAATACGACTCACTATAGGGAGAGGCCATGATATAGACGTTGTGGCTGTT

Table S2: LNA primer, Padlock and detection probe sequences

PLP-rG2FlipTDP1	CTTGCAGTATTGAGAGTGTACCGACCTCAGTAAGTAGCCGTGACTATCGACTTCCCTGAACCCCAGTAAAT
PLP-rG2FlopTDP2	GTTAACCTCGCAGTACTACCTTAGCTACGACCTCAATGCACATGTTTCCATCCTCTTCTCCAGGAAATGCG
DP1-AF555	AGTAGCCGTGACTATCGACT
DP2-AF488	CCTCAATGCACATGTTTCCATCC
LNA G2-Flip	C+TT+TT+CC+TT+AC+TT+CCCGAGTCCTTGGC
LNA G2-Flop	G+GG+CA+CT+GG+TC+TT+TTCCTTGGAATCAC

Supplemental Table S3: Primer sequence for PCR reactions

Target gene	**Name and Primer Sequence**	
rGRIA1	rG1 F	CAGAGGAAGGCATGATCAGAGTGAGAAAA
	rG1 R	CAGCATGGCCAGTCCCAGCCCA
rGRIA2	rG2 R/G EF	ATGAACGAGTACATCGAGCAGAGG
	rG2 R/G ER	CCCCGACAAGGATGTAGAATACTC
	rG2 R/G ER Flip	GTCTTTTCCTTACTTCCCGAGTCCT
	rG2 R/G ER Flop	GTCTTTTCCTTGGAATCACCTCC
	rG2 Q/R EF	GGAATCTCTATCATGATCAAGAAGCC
	rG2 Q/R ER	CCACACACCTCCAACAAT GC
rGRIA3	rG3 R/G EF	TACATTGAGCAGAGAAAGCCGTGC
	rG3 R/G ER	GCAGGAGCAGGCTTAAAGTTTTGG
	rG3 R/G ER Flip	ACTGGTCTTGTCCTTACTCCCGGAG
	rG3 R/G ER Flop	CTTGTCCTTGGAGTCACCGCCC
rGRIA4	rG4 R/G EF	CACGATGAAAGTGGGAGGAAACCTGGAT
	rG4 R/G ER	CCTGGCTTTGTTTCTTATGGCTTCGGAAAAA
	rG4 R/G ER Flip	CGTCTTGTCCTTGCTTCCCGAG
	rG4 R/G ER Flop	ACTCGTCTTGTCCTTGGAGTCACCTC
rβ3-tub	rTubb3-F	AGCGCATCTCCGAGCAGTTT
	rTubb3-R	TGGGCTTCCGACTCCTCGTC
rPSD95	rPSD95-F	GTGGGCGGCGACGATGGTGAA
	rPSD95-R	GATGATCGTCGTCGTACCCG
rFMR1	rFMR1-F	GATGAAGTTGAGGTTTATTCCAGAGC
	rFMR1-R	CGATTCCCCGGCACACATTTCT
αCAMKII	rαCAMKII-F	GAAGCCATTAGCAATGGAGA
	r αCAMKII-R	GGTTTTCAAAATAGAATCGATG
rTF2B	rTF2B-F	CCAGGACATTTAAAGAAATATGTGCT
	rTF2B-R	GTAACATCAGCAACACCAGCAA
rEGFP	EGFP-F	TGAAGCAGCACGACTTCTT
	EGFP-R	GGCCATGATATAGACGTTGTGGCTGTT
rβ-Act	β-Act-F	TAAAGACCTCTATGC CAACACAGT
	β-Act-R	GAGGGGCCGGAC TCATCGTAC
rNG2	NG2-F	ATCTGGGAGGGGGCTATTGT
	NG2-R	GTACGCCATCAGAGAGGTCG
rMBP	MBP-F	GCTGTGCCACATGTACAAGGA
	MBP-R	TAGGCCCCCTTGAATCCCTT
rGFAP	GFAP-F	CCTGGAACAGCAAAACAAGGC
	GFAP-R	CCTCTCCAGATCCACACGAG
rS100	S100-F	TTGGACACCGAAGCCAGAGA
	S100-R	TCAGCTTGTGCTTGTCACCC

Supplementary Results

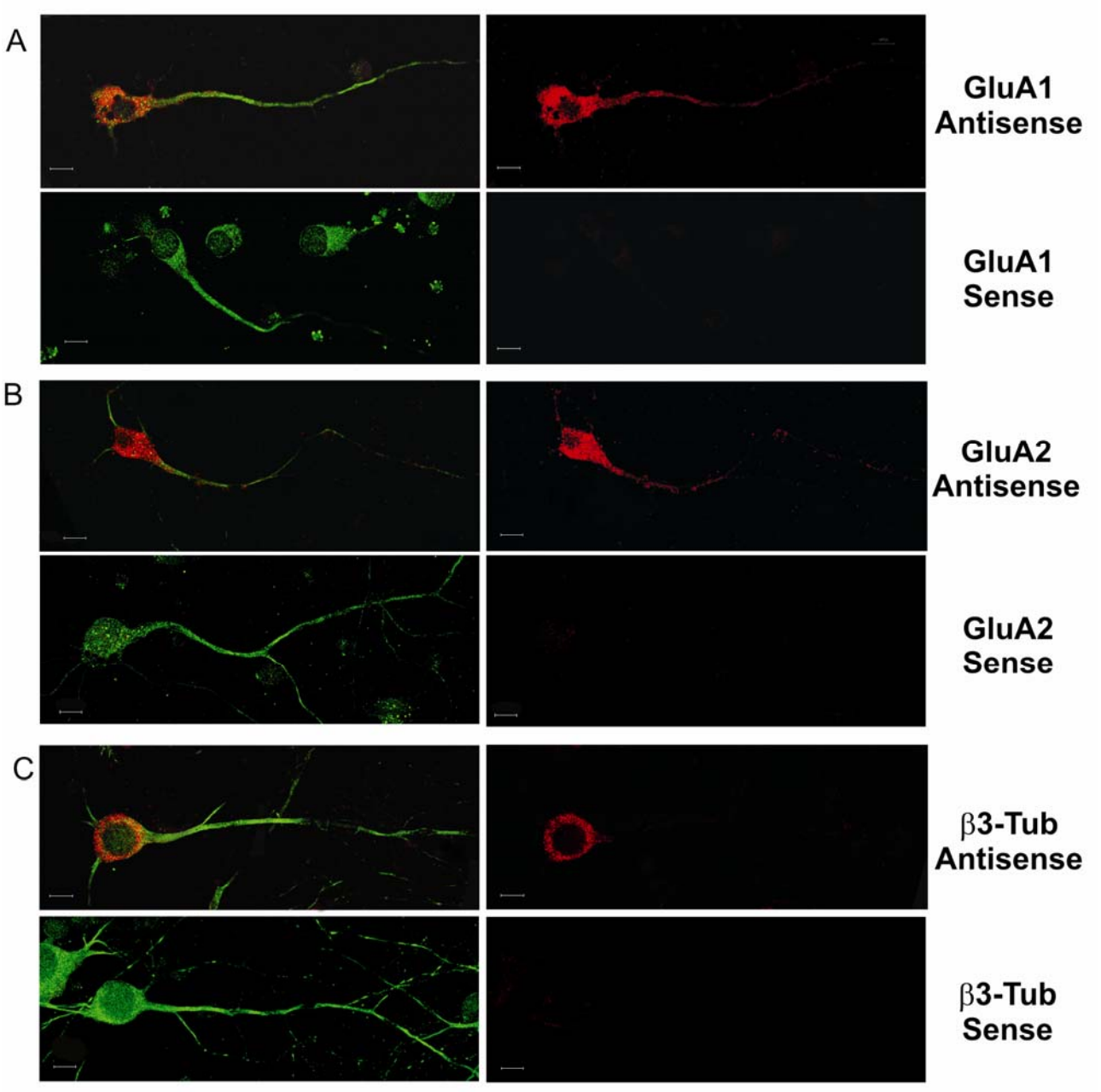


Figure S1: Fluorescence *in situ* hybridization of GluA1, GluA2 and β3-Tub mRNAs in primary cortical neurons. The right panel shows the hybridization signal (red) of the different mRNAs. In the left panel, the figures are merged with the immunostaining for Microtubule Associate Protein 2 (MAP2). (A) Fluorescence *in situ* hybridization using GluA1 antisense and sense probes. (B) Fluorescence in situ hybridization using GluA2 antisense and sense probes. (C) Fluorescence in situ hybridization using β3-Tub antisense and sense probes. Scale bar 10 μm. As reported, **the different sense probes did not show any hybridization signal**.

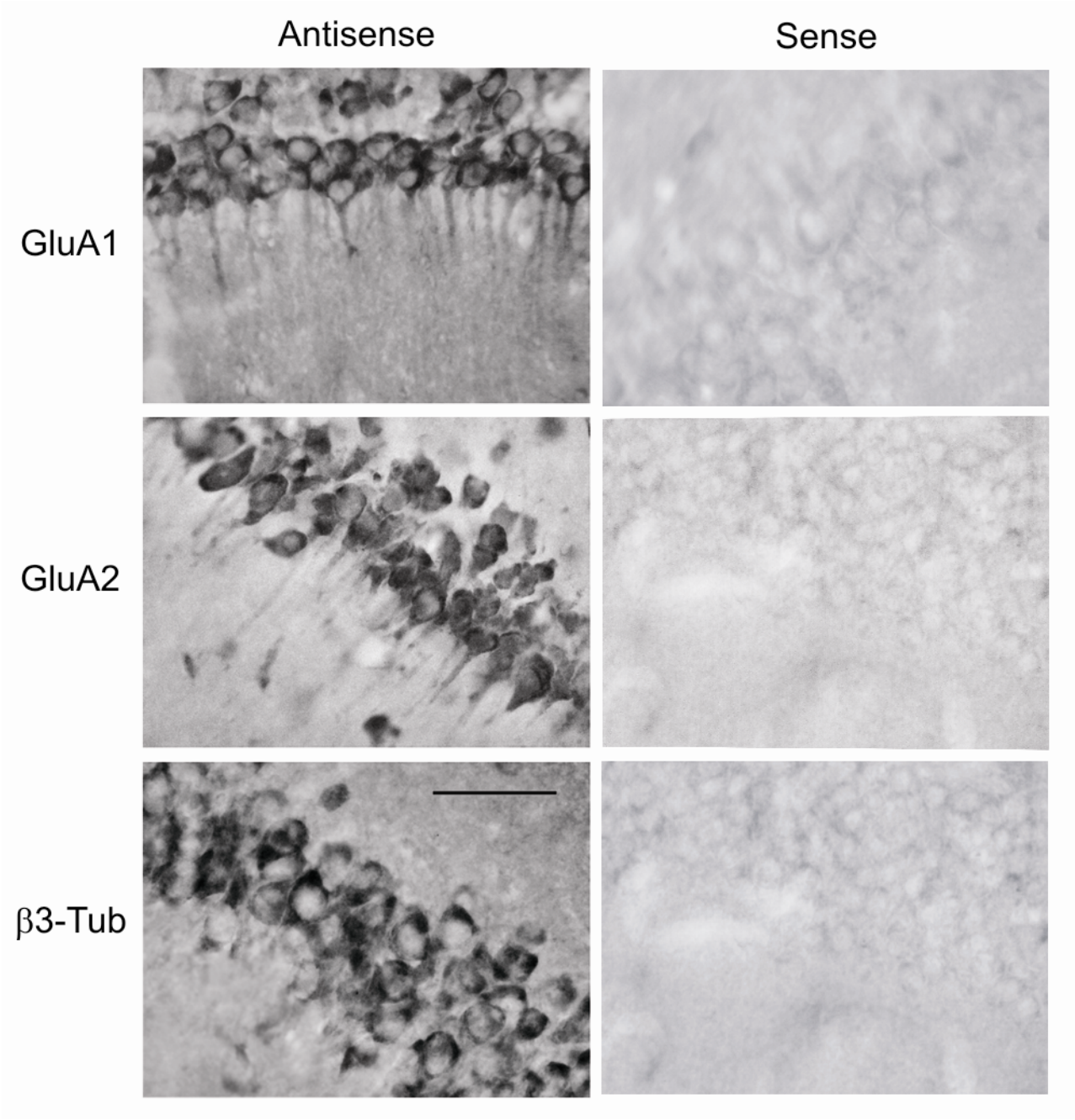


Figure S2: In situ hybridization with alkaline phosphatase detection of GluA1, GluA2 and β3-Tub mRNAs in rat hippocampus slices. Left panel shows the antisense hybridization signal; right panel shows the slices hybridized with sense ribobrobes. Scale bar 50 μm. **The different sense probes did not show detectable hybridization signal**

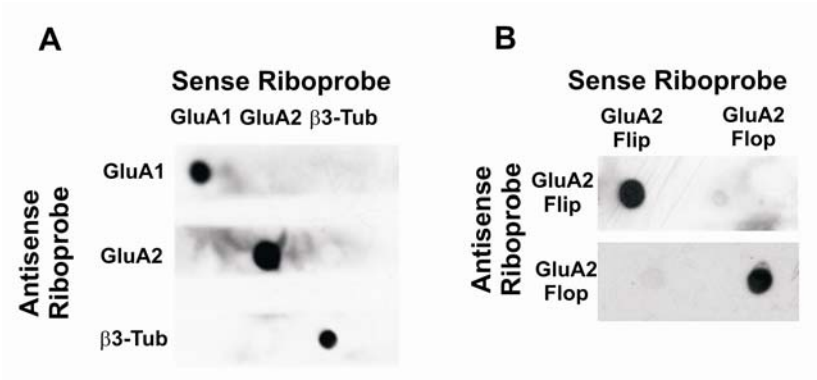


Figure S3: Reverse dot blot**.**

Nylon membranes were spotted with (A) GluA1, GluA2 and β3-Tub (B) GluA2 Flip and GluA2 Flop sense probes, UV cross-linked and hybridized with DIG label GluA1, GluA2 and β3-Tub antisense probes (A) and GluA2 Flip and GluA2 Flop antisense probes (B) in separate reactions. Chemiluminescent signals were detected after incubation with anti-digoxigenin (DIG)-AP coniugated antibody and CDP-star. **The results show the specificity of antisense probes for their definite target sequences without cross-hybridization with other sequences**.

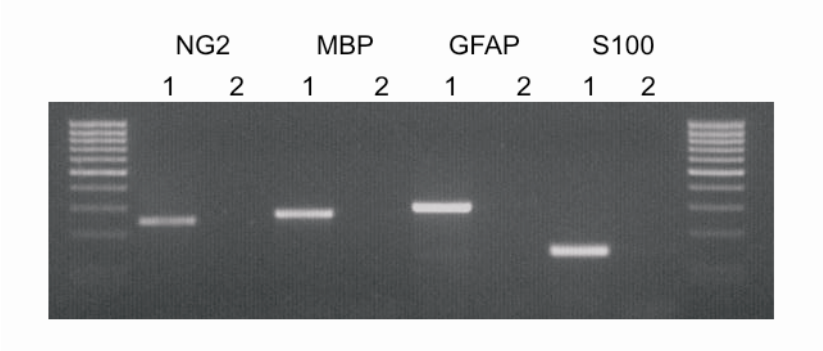


Figure S4: Glial Markers

To test if synaptoneurosomal preparation might be contaminate by cell of glial origin we amplify several glial markers, such as Nerve Glia antigen 2 (NG2), Myelin Basic Protein (MPB), S100 calcium-binding protein, Glial Fibrillary Acidic Protein (GFAP), from both total (1) and SNS (2) preparation. No amplification product can be observed in SNS for any of the markers used. **These data confirm that AMPA receptor mRNAs in SNS derive mainly from neuronal cells.**

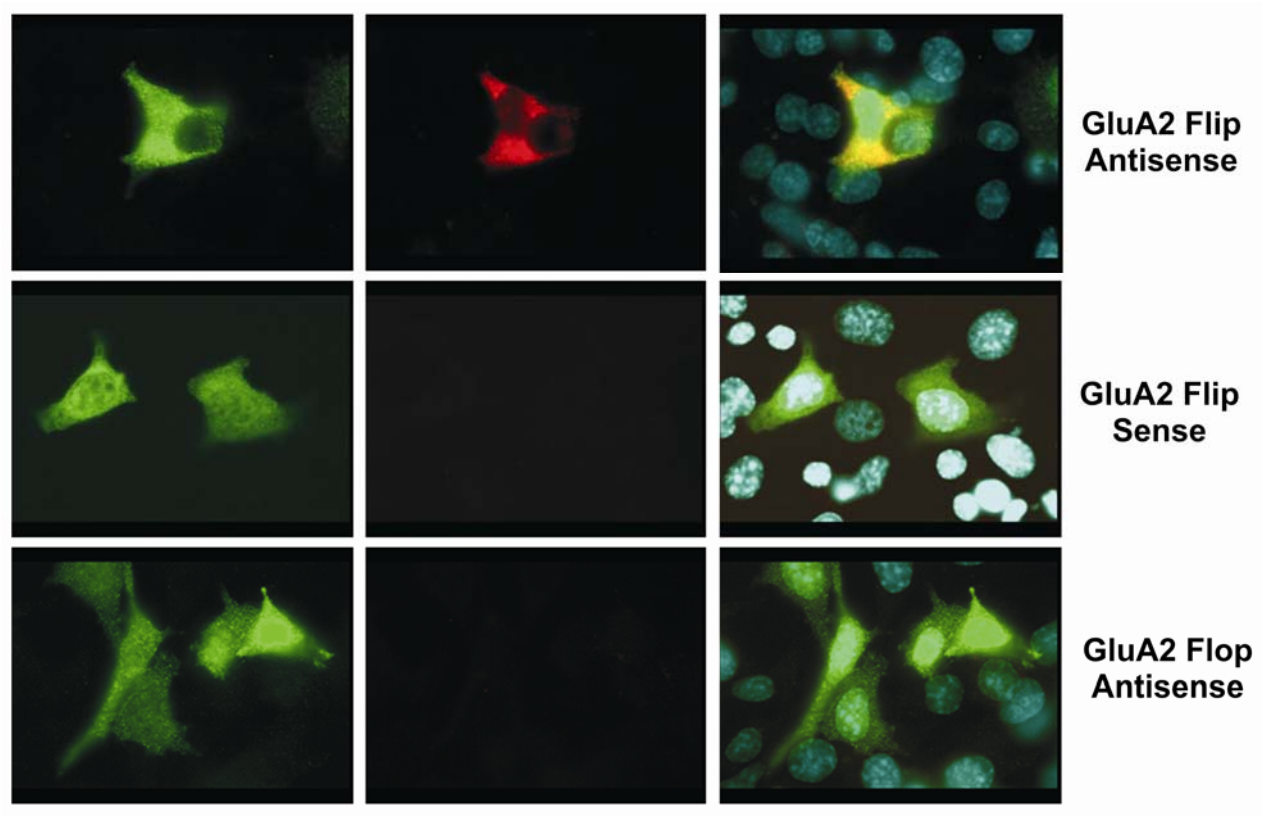


Figure S5: GluA2 Flip Riboprobe sequence specificity

PC12 cells were transfected using a pEGFP DNA based vector expressing GluA2 Flip RNAs. The cells were then hybridized using the antisense (AS) riboprobes for the Flip sequence, the sense (S) riboprobes for the Flip sequence, and AS riboprobe detecting the Flop sequence. The left panels shows the EGFP fluorescent signal (green), middle panel show RNA in situ hybridization signal (red), in the right panel the two signals are merged and the nuclei are stained with DAPI. **These experiments showed that the Flip AS probe hybridizes to its own target RNA, while the S probe does not report any signal, confirming the specificity of the protocol used. Moreover, the Flop AS probe does not hybridize on GluA2 Flip RNAs showing probe specificity.**

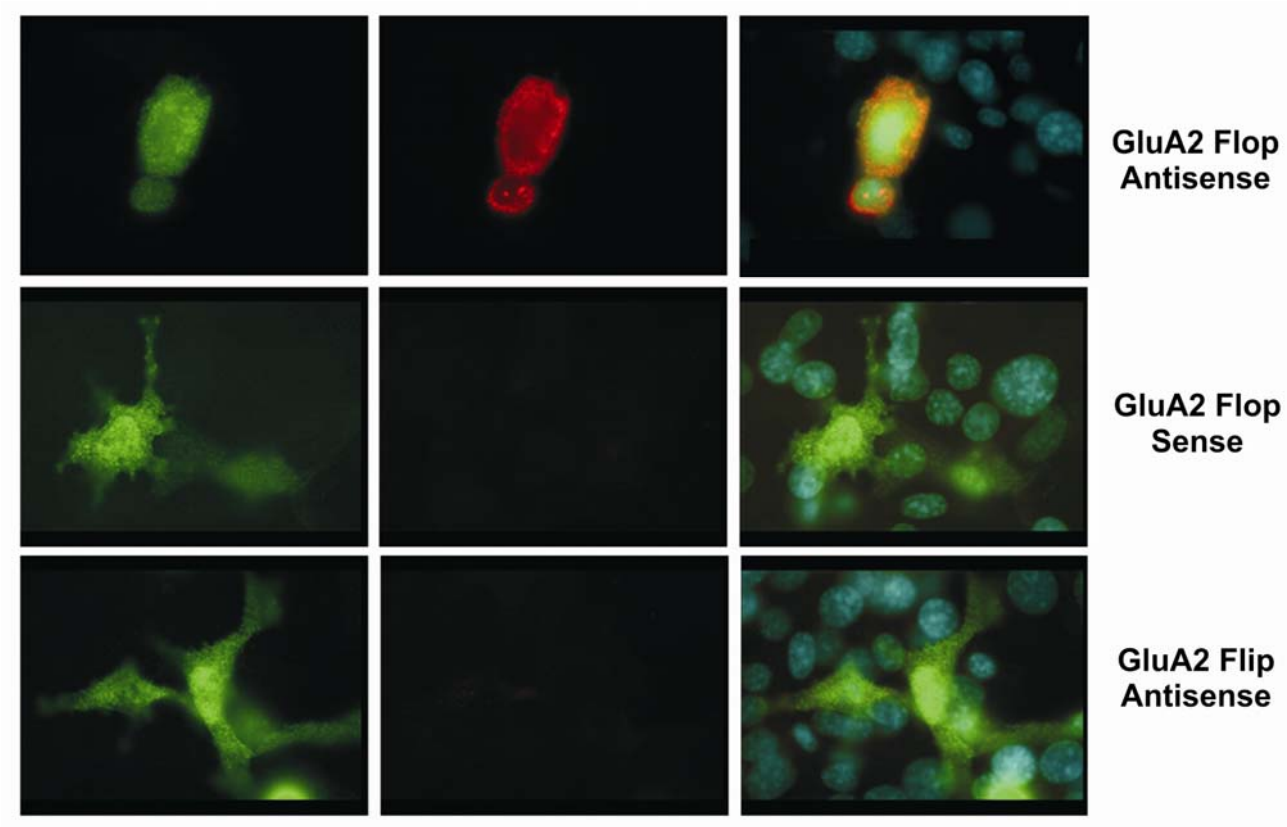


Figure S6- GluA2 Flop Riboprobe sequence specificity

PC12 cells were transfected using a pEGFP DNA based vector expressing GluA2 Flop RNAs. The cells were then hybridized using the antisense (AS) probes for the Flop sequence, the sense (S) probes for the Flop sequence, and the AS probe detecting the Flip sequence. The left panels shows the EGFP fluorescent signal (green), the middle panel show RNA in situ hybridization signal (red), in the right panel the two signals are merged and the nuclei are stained with DAPI. **These experiments showed that the Flop AS probe hybridizes to its own target RNA, while the S probe does not report any signal, confirming the specificity of the protocol used. Moreover, the Flip AS probes do not hybridize on GluA2 Flop RNAs, showing probe selectivity**.

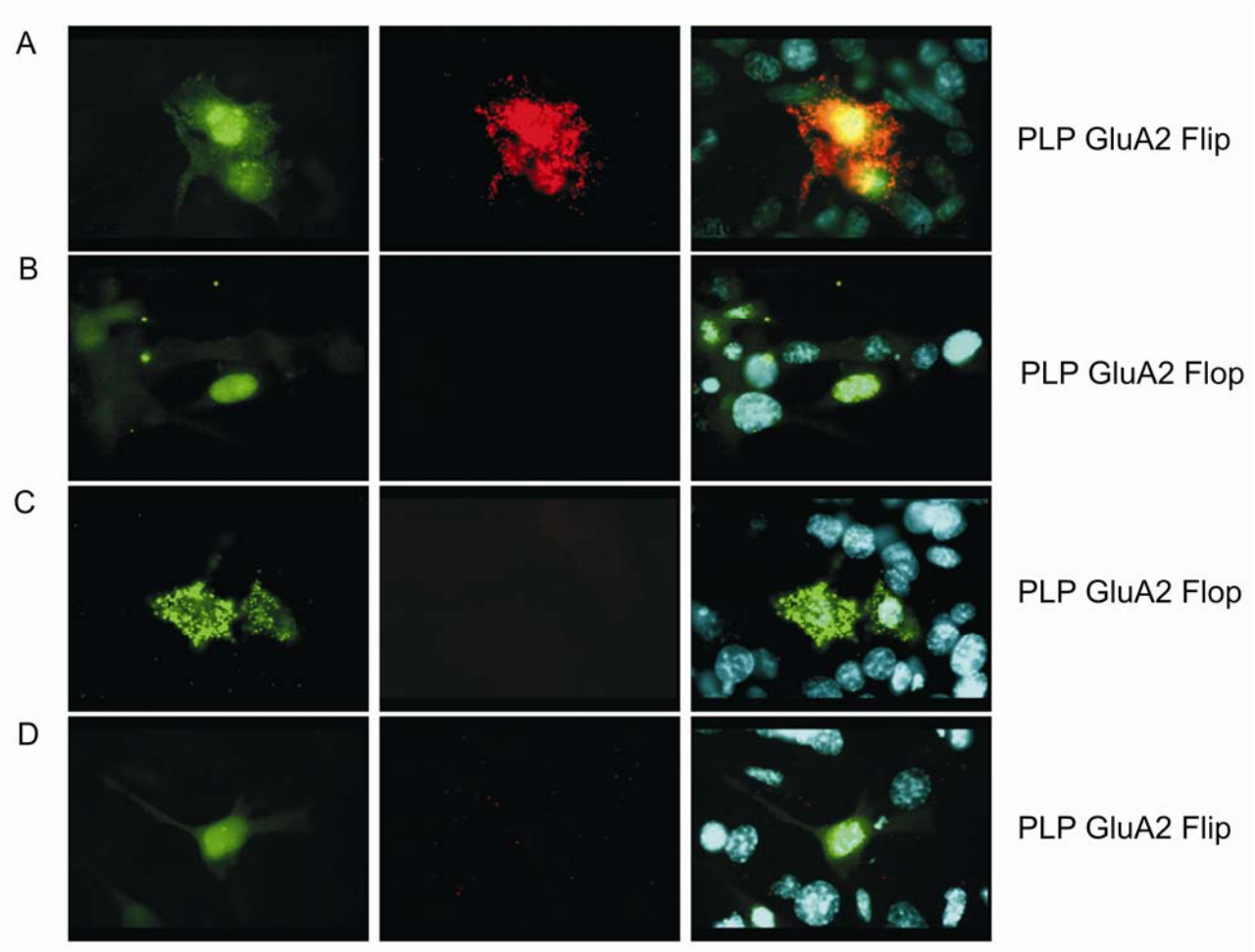


Figure S7 Padlock Probes hybridization specificity

PC12 cells were transfected using a pEGFP DNA based vector expressing GluA2 Flip RNAs (A and B) and GluA2 Flop RNAs (C and D); in these vectors the EGFP sequences were preceded by a sequence coding for a Nuclear Localization Signal that induced the EGFP protein to localize mainly in the nucleus, so that the EGFP fluorescence signal do not interfere with signals deriving from Padlock probes. After transfection, the cells were subjected to hybridization using Padlock Probe (PLP) specific for the GluA2 Flip sequence (A and D) and GluA2 Flop sequence (B and C). Corrected hybridization of GluA2 Flip sequence gives a red spotted signal (in the middle panels) while that of Flop sequence gives a green spotted signal (in the left panels). EGFP signal stains the nucleus with a homogeneous green signal (left panels).

As reported, GluA2 Flip transfected cells (A, B) showed clear RCPs (Rolling Cycle Particles) when detected with Flip PLP (A -middle panel- red spotted signal) but not with Flop PLP (B -left panel- no green spotted signal). On the other hand, GluA2 Flop transfected cells (C, D) showed clear RCPs when detected with Flop PLP (C- left panel- green spotted signal) but not with Flip PLP (D- middle panel- no red spotted signal). **These data showed that there is not cross hybridization between the two PLPs used in our protocol**.

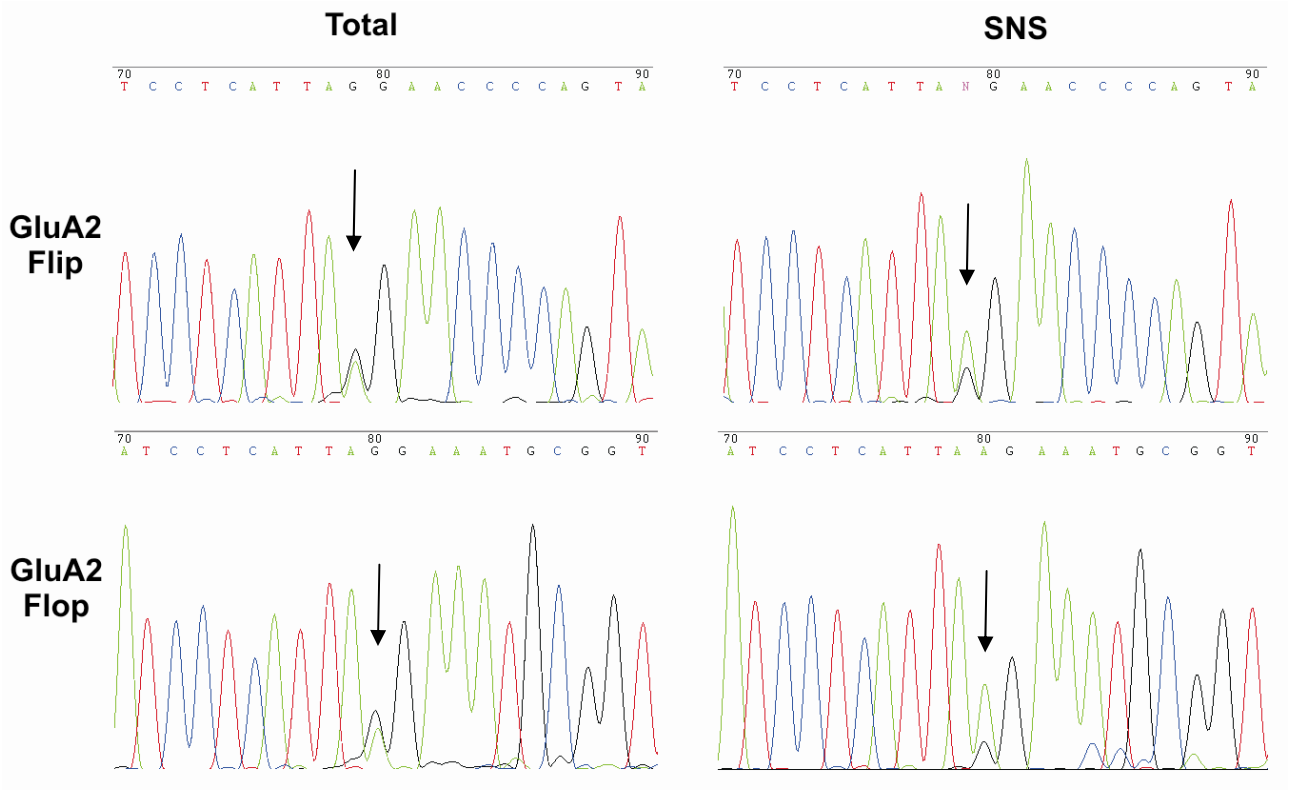


Figure S8: Editing analysis

Representative examples of GluA2 Flip and Flop electropherograms used to calculate the R/G editing level for the Flip and the Flop isoform in total and synaptoneurosomal (SNS) preparations. The nucleotide subjected to editing (R/G site, arrowed) is reported as two overlapping peaks, the editing level is calculated with DS genes analysis software as reported in material and methods; the protocol as been previously standardized (3-5)

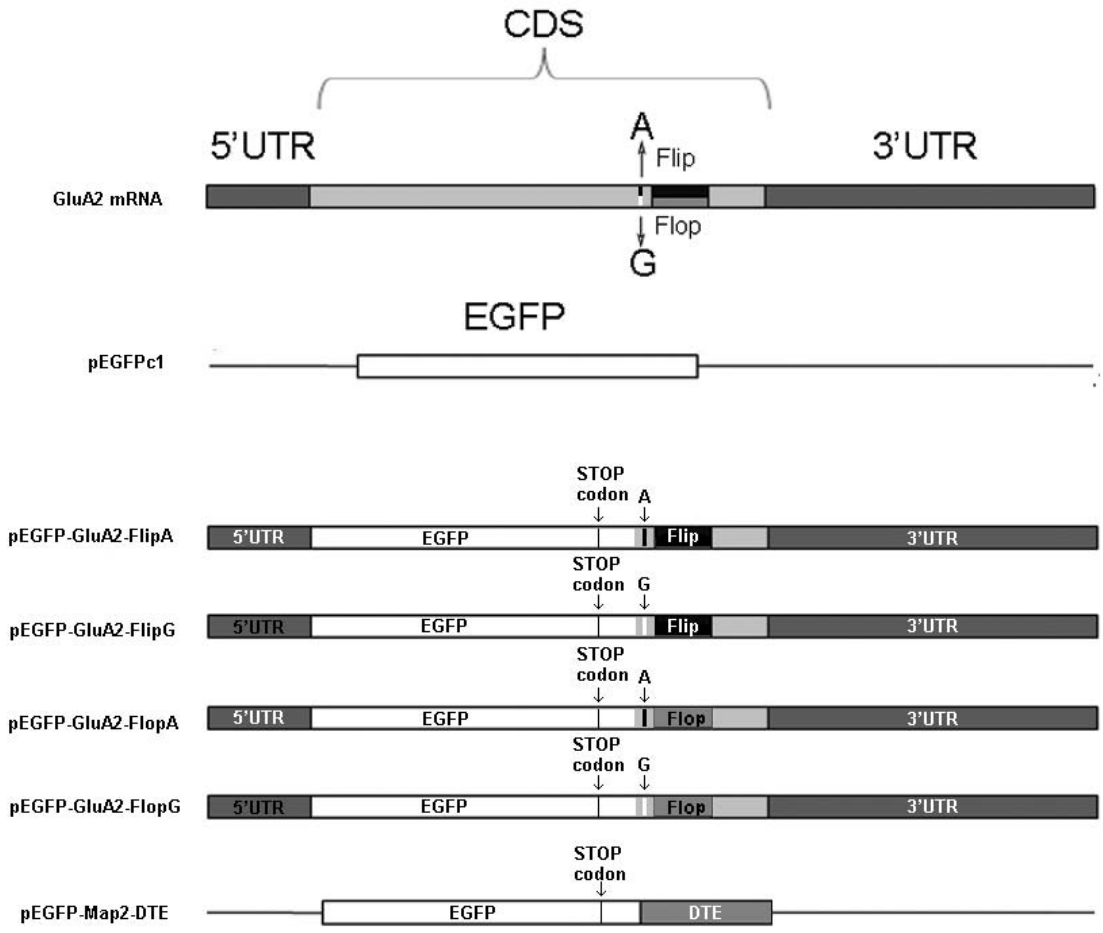


Figure S9 Schematic representation of DNA constructs used in transfection experiments.

The 5'UTR of GluA2 (nucleotide 1-433-ATG) was inserted upstream of the EGFP cds, using NheI/AgeI restriction sites. Downstream EGFP coding sequence, using PstI/SacII enzymes in the multicloning sites, the sequences of GluA2 last three exons (including the R/G editing site and Flip or Flop exons) plus 2524 nts of the 3'UTR were inserted. Due to the addition of a stop codon after the EGFP cds, GluA2 sequence is not translated but only transcribed into chimeric mRNA molecules. Therefore, every DNA construct, while producing different chimeric RNAs, express only the EGFP that is use to detect transfected cells. As a control the Dendritic Target Element (DTE) of Map2 was inserted downstream EGFP sequence using PstI/SacII resctriction sites.

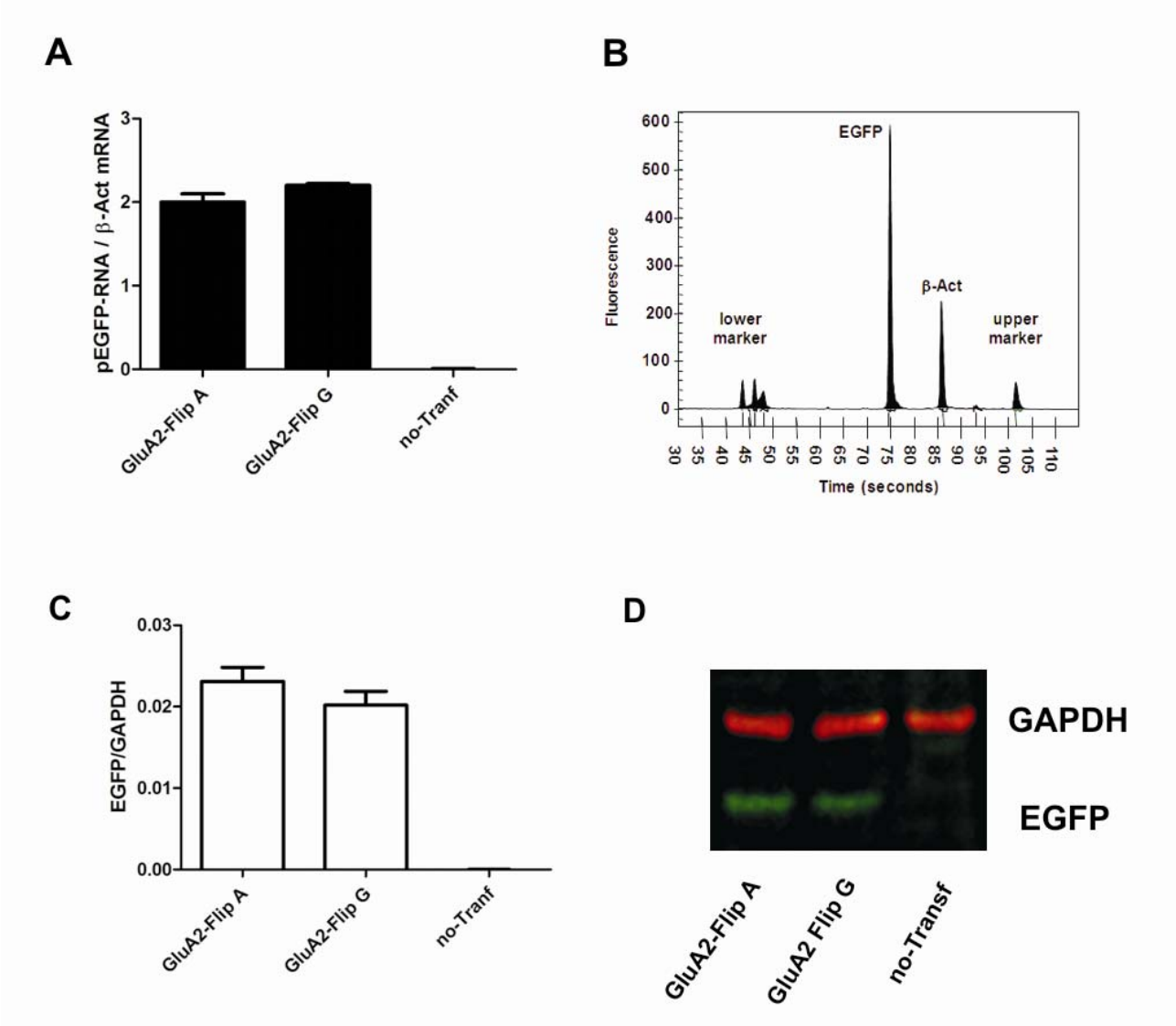
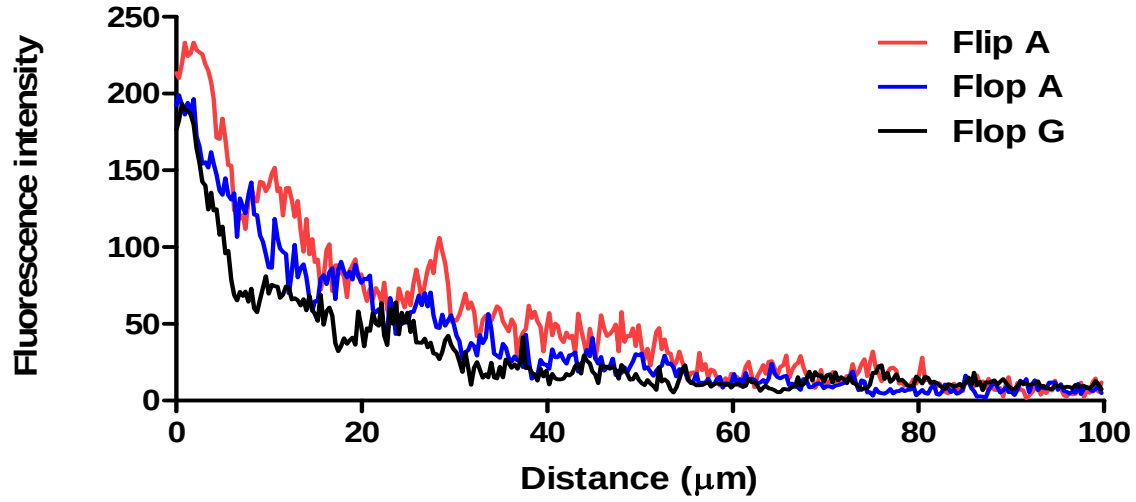


Figure S10: Transcriptional and translational efficiency of the pEGFP DNA constructs.
To determine the transcription efficiency of the different DNA vectors used in transfection esperiments, co-amplifications of EGFP RNA and β-Actin RNA were performed from PC12 transfected cells. PCR products were analyzed by means of the Agilent Bioanalyzer technology. (A) Histogram showing the relative expression levels of the DNA constructs expressing GluA2-FlipA and GluA2-FlipG that presented a similar level of expression; non-transfected cells had no expression of EGFP mRNA. (B) Example of the electropherogram window of EGFP/β-Act co-amplification. The window reported the fluorescence peak intensity of the EGFP and β-Act PCR products; the upper and lower markers are used as internal standards to eliminate sample-to-sample variation, giving reproducibility to the system. The software calculates the size and the concentration of the PCR products comparing their fluorescence intensities with those of the internal standards.
(C) Relative EGFP protein expression for GluA2-FlipA and GluA2-FlipG DNA vector. Non-transfected cells showed no expression of EGFP protein. (D) Representative western blot showing GAPDH and EGFP protein expression.
These experiments showed that the GluA2-FlipA and GluA2-FlipG vector used in RNA localization experiments have the same transcriptional and translational efficiency.

A



B

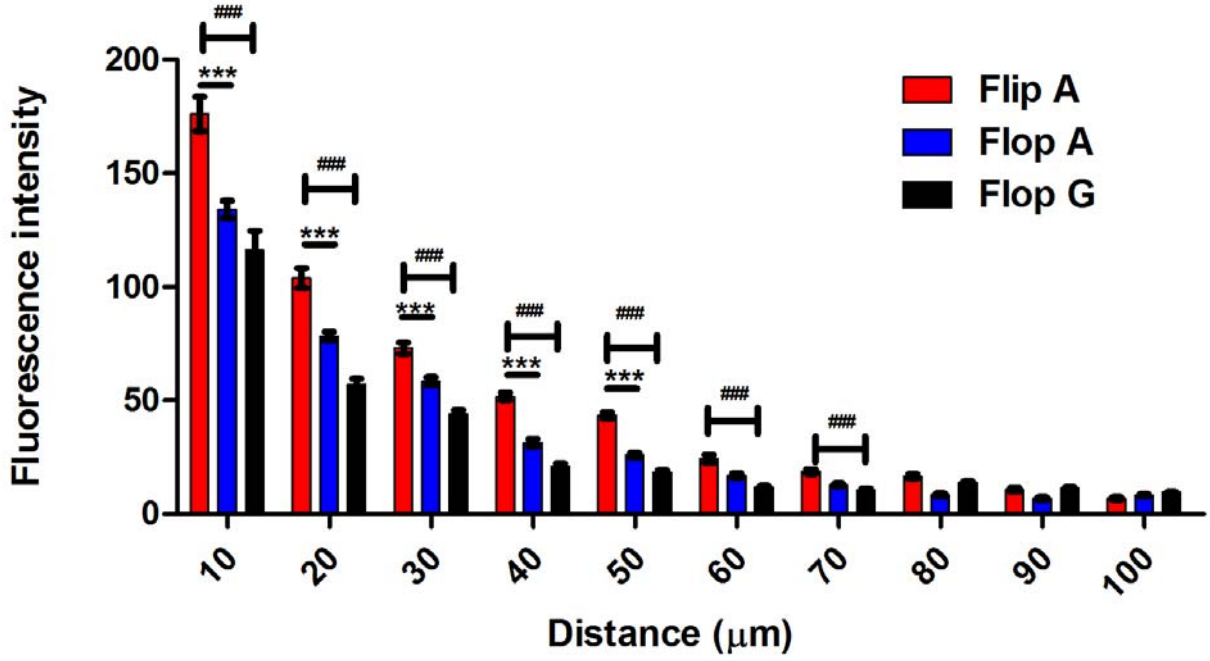


Figure S11. Histograms showing the quantification of GluA2-FlopA and GluA2-FlopG chimeric transcripts dendritic localization; quantification of GluA2-FlipA is reported as reference. (A) The mean fluorescence intensity in dendrites up to 100 μm from the cell soma has been reported for the different chimeric mRNAs ($n \geq 20$). (B) Mean fluorescence intensity in dendritic regions of 10 μm in length, up to 100 μm from the cell soma. Statistical analysis has been dane with two-way ANOVA, followed by Bonferroni post test. FlipA vs FlopA *** $p<0.001$; FlipA vs FlopG ### $p<0,001$; ($n \geq 20$).

References

1. Mueller, O., Hahnenberger, K., Dittmann, M., Yee, H., Dubrow, R., Nagle, R. and Ilsley, D. (2000) A microfluidic system for high-speed reproducible DNA sizing and quantitation. *Electrophoresis*, **21**, 128-134.
2. Barbon, A., Popoli, M., La Via, L., Moraschi, S., Vallini, I., Tardito, D., Tiraboschi, E., Musazzi, L., Giambelli, R., Gennarelli, M. *et al.* (2006) Regulation of editing and expression of glutamate alpha-amino-propionic-acid (AMPA)/kainate receptors by antidepressant drugs. *Biol Psychiatry*, **59**, 713-720.
3. Barbon, A., Vallini, I., La Via, L., Marchina, E. and Barlati, S. (2003) Glutamate receptor RNA editing: a molecular analysis of GluR2, GluR5 and GluR6 in human brain tissues and in NT2 cells following in vitro neural differentiation. *Brain research*, **117**, 168-178.
4. Barbon, A., Fumagalli, F., Caracciolo, L., Madaschi, L., Lesma, E., Mora, C., Carelli, S., Slotkin, T.A., Racagni, G., Di Giulio, A.M. *et al.* (2010) Acute spinal cord injury persistently reduces R/G RNA editing of AMPA receptors. *Journal of neurochemistry*, **114**, 397-407.
5. Barbon, A., Orlandi, C., La Via, L., Caracciolo, L., Tardito, D., Musazzi, L., Mallei, A., Gennarelli, M., Racagni, G., Popoli, M. *et al.* (2011) Antidepressant treatments change 5-HT2C receptor mRNA expression in rat prefrontal/frontal cortex and hippocampus. *Neuropsychobiology*, **63**, 160-168.