SUPPLEMENTAL INFORMATION

SUPPLEMENTAL DATA

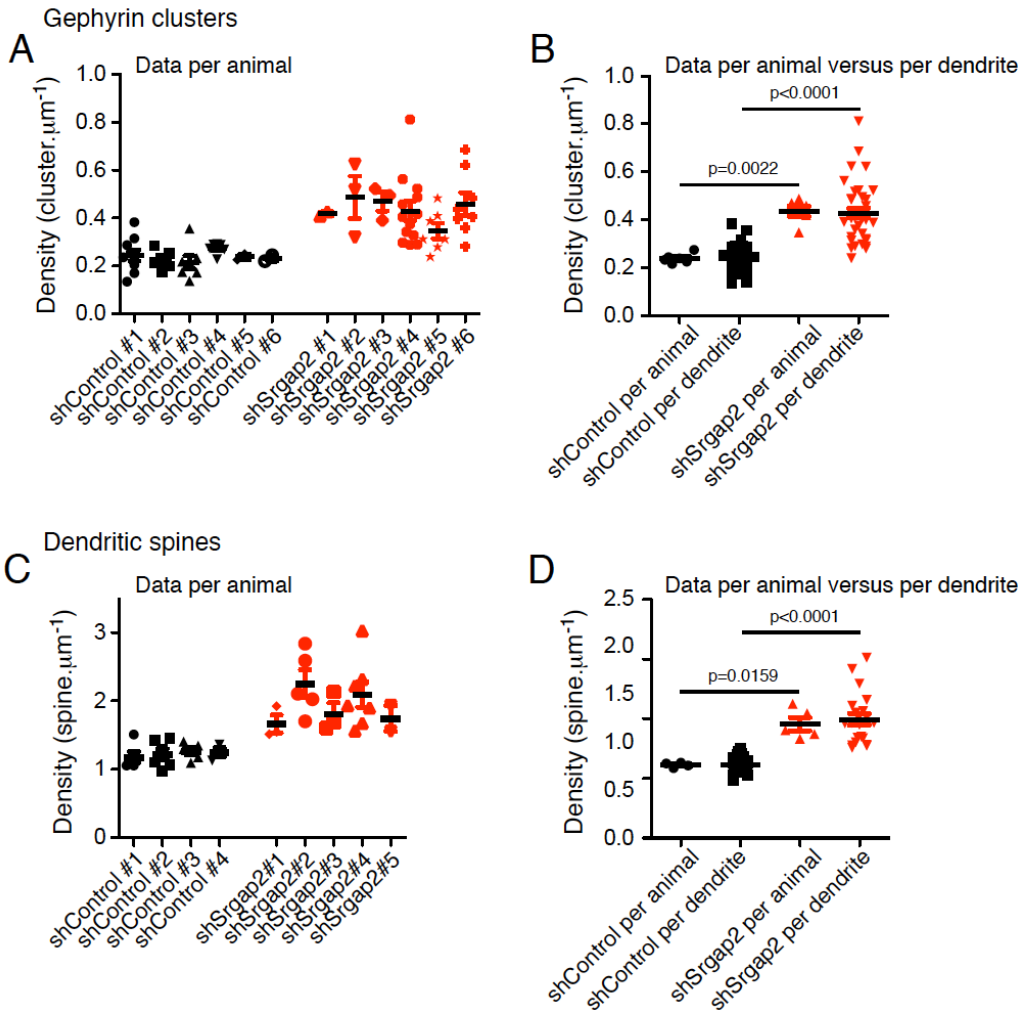


Figure S1. Analysis of shSrgap2 effects on dendritic spine and gephyrin cluster density per animal (related to Figures 1 and 3).

(A) Plot showing the density of gephyrin clusters per dendrite for each mouse that was analyzed in control condition (shControl) and after *Srgap2a* knock-down (shSrgap2) at P21 (same data as in Figure 1). Each dot represents one dendrite, the bars indicate the mean ± SEM.

(B) Comparison of the distribution of gephyrin cluster density when the dendrites are analyzed individually or when the data are averaged per animal. #: mouse identification. Animals: $n_{shControl} = 6$, $n_{shSrgap2} = 6$, dendrites: $n_{shControl} = 32$; $n_{shSrgap2} = 36$, Mann-Whitney test.

(C) Plot showing the density of dendritic spines per dendrite for each mouse that was analyzed in shControl and after shSrgap2 at P21 (same data as in Figure 3). Each dot represents one dendrite, the bars indicate the mean ± SEM.

(D) Comparison of the distribution of dendritic spine density when the dendrites are analyzed individually or when the data are averaged per animal. Animals: $n_{shControl} = 4$, $n_{shSrgap2} = 5$, dendrites: $n_{shControl} = 19$; $n_{shSrgap2} = 20$, Mann-Whitney test.
Note that the variability is greater between dendrites than between animals but the mean values are similar.

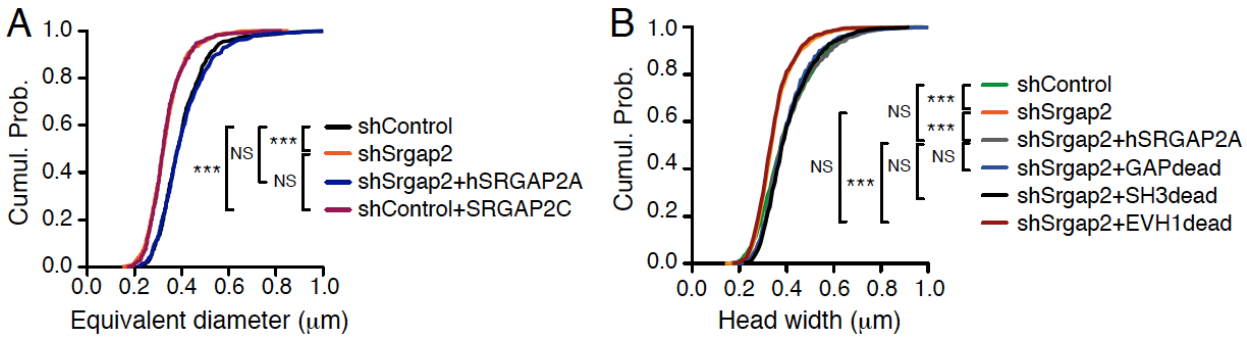


Figure S2. Distribution of Gephyrin cluster and dendritic spine head size in juvenile cortical pyramidal neurons following *in utero* manipulation of SRGAP2 paralogs (related to Figures 1 and 3).

(A) Cumulative probability plot of the equivalent diameter of individual Gephyrin clusters in the indicated conditions. Data come from the P21 layer 2/3 cortical pyramidal neurons quantified in Figure 1 (shControl, shSrgap2, and SRGAP2C) and Figure 4 (shSrgap2+hSRGAP2A). $n_{shControl}$ = 537, $n_{shSrgap2}$ = 803, $n_{shSrgap2+hSRGAP2A}$ = 512, $n_{shControl+SRGAP2C}$ = 768.

(B) Cumulative probability plot of individual spine head width in the indicated conditions. Data come from the P21 Layer 2/3 cortical pyramidal neurons quantified in Figure 3. $n_{shControl}$ = 1,223; $n_{shSrgap2}$ = 1,334; $n_{shSrgap2+hSRGAP2A}$ = 871; $n_{shSrgap2+GAPdead}$ = 1,582; $n_{shSrgap2+SH3dead}$ = 1,056; $n_{shSrgap2+EVH1dead}$ = 876 . *** $p < 0.001$, NS: $p > 0.05$, Kruskal-Wallis test followed by the Dunn's Multiple comparison test.

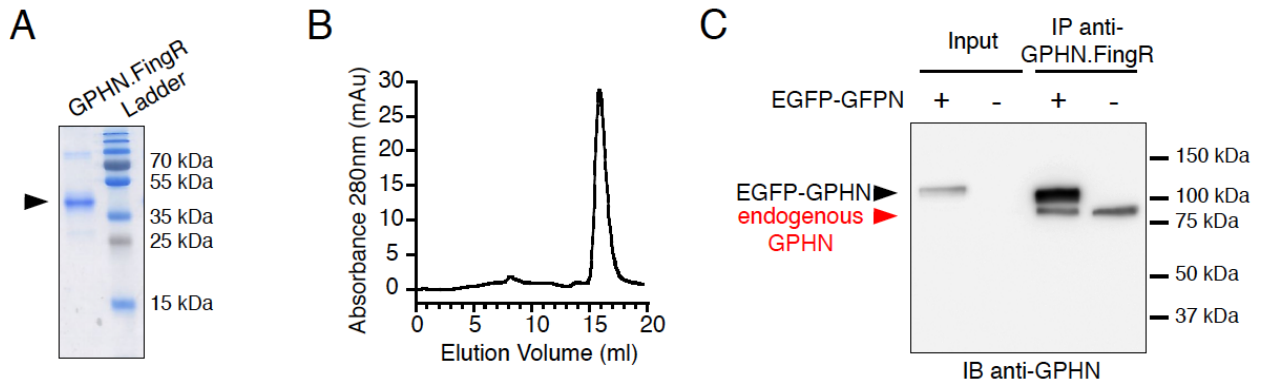


Figure S3. Purification and characterization of GPHN.FingR (related to Figure 2).

(A) SDS-PAGE stained with Coomassie Blue. Left lane: 10 µl of purified GPHN.FingR tagged with EGFP, His and Flag were loaded, right lane: prestained protein ladder. Purified GPHN.FingR (arrowhead) migrates at its expected molecular weight (44 kDa).

(B) Gel filtration chromatogram (calibrated S200, GE Healthcare) of purified GPHN.FingR shows a single peak at 15.8 ml, which is consistent with a 44 kDa globular protein.

(C) Purified GPHN.FingR immunoprecipitates Gephyrin (Western blot). GFP-GPHN was overexpressed in HEK cells. 1 mg of cell lysate was incubated overnight in the presence of 25 µl of GPHN.FingR. Anti-Flag M2-coupled magnetic beads were subsequently added for 2 hr. After extensive washing, bound proteins were eluted by boiling in loading buffer. Note that GPHN.FingR pulled down both overexpressed EGFP-GPHN (black arrowhead) and endogenous Gephyrin (red arrowhead), although endogenous Gephyrin was hardly detectable in the input. Gephyrin was not immunoprecipitated in the absence of GPHN.FingR (see Figure 2D).

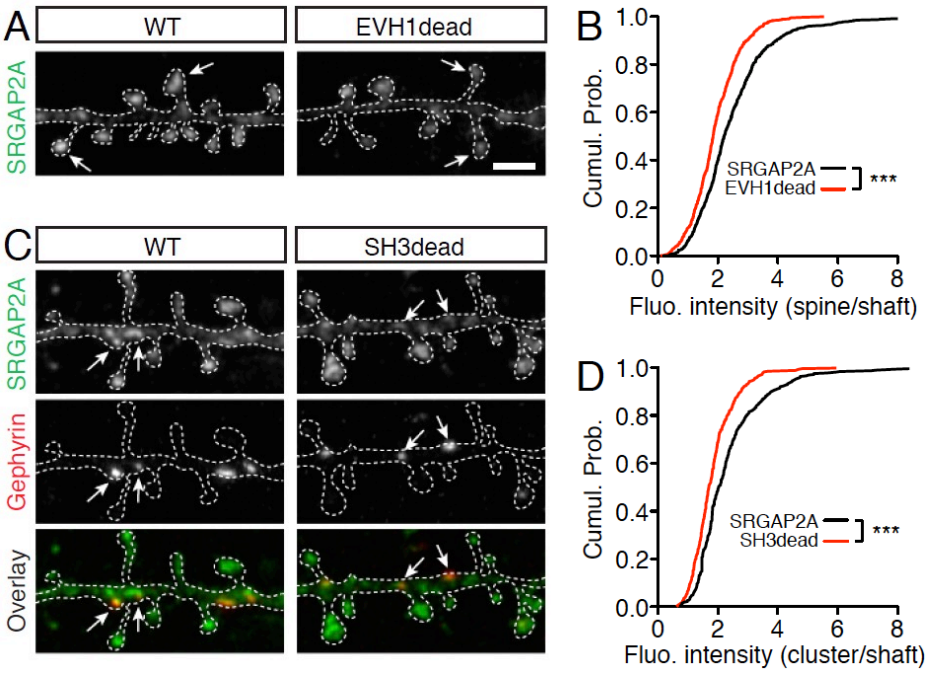


Figure S4. Reduced localization of SRGAP2A mutants to excitatory and inhibitory synapses (related to Figure 2).

(A) Confocal images of cultured cortical neurons expressing SRGAP2A-GFP (left) or EVH1dead-GFP (right). Dashed lines indicate the contours of spines and dendrites defined by the fluorescence associated with soluble blue fluorescent protein (BFP). Scale bar = 2 μm.

(B) Cumulative probability plot of GFP fluorescence intensity associated with spines for SRGAP2A-GFP and EVH1dead-GFP. The signal was normalized to the GFP fluorescence in the corresponding dendrites (ratio spine/shaft). $n_{SRGAP2A}$ = 905 spines, 18 neurons; $n_{EVH1dead}$ = 615 spines, 11 neurons; *** $p < 0.0001$, Mann-Whitney test.

(C) Confocal images of dissociated cortical neurons expressing SRGAP2A-GFP or SH3dead-GFP (green) and tdTomato-Gephyrin (red) along with soluble BFP (delineated by the dashed line).

(D) Cumulative probability plot of GFP fluorescence intensity associated with Gephyrin clusters for SRGAP2A-GFP and SH3dead-GFP. The signal was normalized to the GFP fluorescence in the corresponding dendrites outside of Gephyrin clusters (ratio cluster/shaft). $n_{SRGAP2A}$ = 330 clusters, 12 neurons; $n_{SH3dead}$ = 300 clusters, 12 neurons; *** $p < 0.0001$, Mann-Whitney test.

Cortical neurons were *ex utero* electroporated (EUE) at E15.5 with the indicated constructs and shSrgap2 to prevent an overexpression phenotype. The shRNA vector also enabled the expression of soluble BFP and was named pH1SCBFP. The electroporation settings were: 5 pulses of 20 V for 100 ms with 200 ms intervals. Neurons were dissociated and plated immediately after EUE and were imaged after 20 days of culture. For spine quantification, the BFP signal was used to draw ROIs around spine heads that were clearly delineated from the dendritic shaft. For Gephyrin cluster analysis, ROIs were drawn around Gephyrin clusters defined by TdTomato fluorescence. The fluorescence associated with SRGAP2A-GFP and indicated GFP-tagged mutants was measured in these ROIs and normalized to the GFP signal in ROIs of similar size in the same dendritic branch (spine to shaft ratio), and without Gephyrin clusters (cluster to shaft ratio).

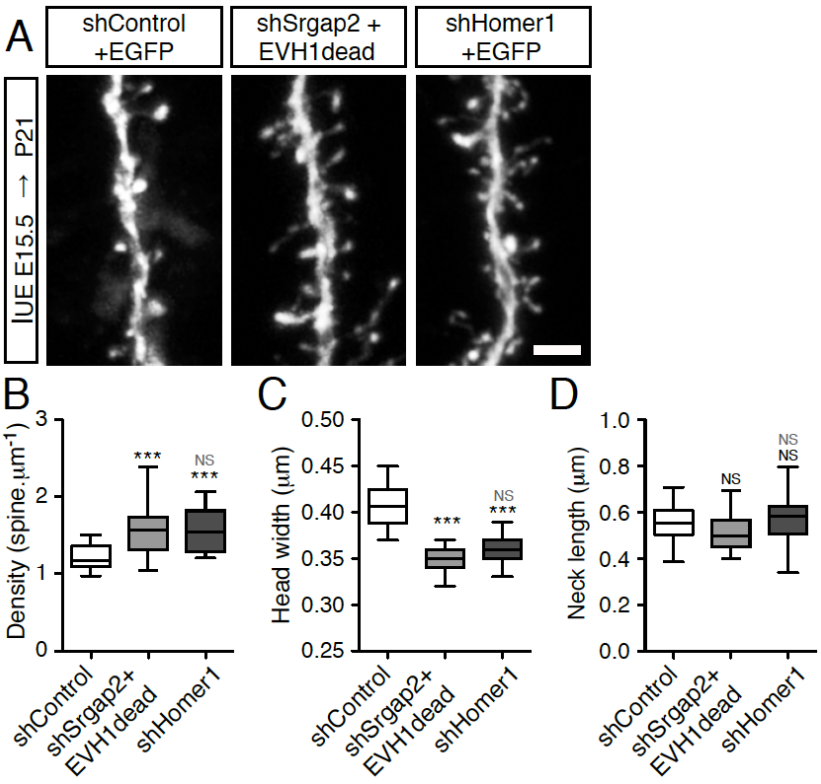
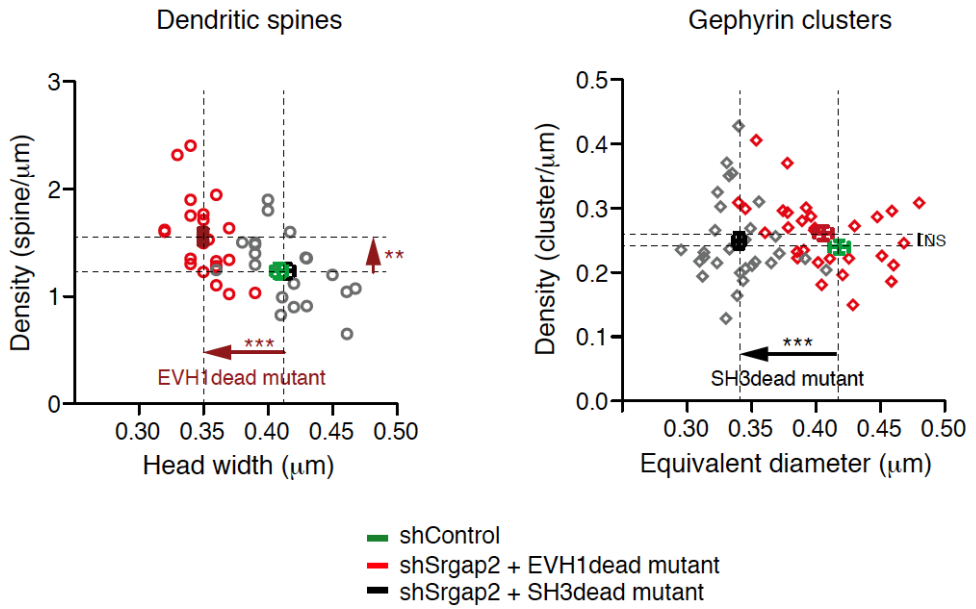


Figure S5. Disruption of SRGAP2A-Homer interaction mimics Homer1 deficiency during spine development *in vivo* (related to Figure 3).

(A) Segments of dendrites from layer 2/3 pyramidal neurons (P21) in control condition (shControl), after *in utero* replacement of mouse *Srgap2a* by SRGAP2A EVH1dead mutant or after Homer1 knock-down (shHomer1). ShHomer1 used the seed sequence 5′-CTG ACC AGT ACC CCT TCA C-3′ and was under H1 promoter in pH1SCV2 vector. Targeting this sequence was previously shown to disrupt Homer1 clusters in hippocampal neurons (Hayashi et al., 2009). In HEK cells, shHomer1 decreased GFP-Homer1c expression to 60% of the control level (data not shown). Scale bar: 2 µm.

(B-D) Distribution of spine density (B), spine head width (C) and mean spine neck length (D). $n_{shControl}$ = 19 (same as in Figure 3), $n_{shSrgap2+hEVH1dead}$ = 24 (same as in Figure 3), $n_{shHomer1}$= 20. *** $p < 0.001$, NS: $p > 0.05$, Kruskal-Wallis test followed by Dunn's Multiple comparison test.



	Density		Size	
	Spines	Gephyrin	Spines	Gephyrin
EVH1dead mutant	↗	→	↘	→
SH3dead mutant	→	→	→	↘

Figure S6. Absence of compensatory regulation of excitatory and inhibitory synapses following disruption of SRGAP2A-Gephyrin and SRGAP2A-Homer interaction, respectively (related to Figures 3 and 4).

Plot (top) and summary table (bottom) showing the effects on dendritic spines (left) and gephyrin clusters (right) of the EVH1dead mutant (red) and SH3dead mutant (grey) compared to hSRGAP2A (control, green) expressed in replacement of endogenous SRGAP2A in sparsely electroporated neurons of juvenile mice. Circles and diamonds represent the average size and corresponding density of dendritic spines (circles) and gephyrin clusters (diamond) for each dendrite analyzed. Bars correspond to the mean value ± SEM for each condition. For clarity, the control condition is only represented as mean ± SEM. *** $p < 0.001$, ** $p < 0.01$, NS: $p > 0.05$, Kruskal-Wallis test followed by the Dunn's Multiple comparison test. Note that the EVH1dead mutant affects the size and density of dendritic spines but not gephyrin clusters. On the contrary, the SH3dead mutant affects the size of gephyrin clusters but does not modify dendritic spines.

	Gephyrin clusters	Dendritic spines
shControl (juvenile)	Mouse #1: 10 Mouse #2: 5 Mouse #3: 7 Mouse #4: 6 Mouse #5: 2 Mouse #6: 2	Mouse #1: 5 Mouse #2: 6 Mouse #3: 5 Mouse #4: 5 Mouse #5: 3
shSrgap2 (juvenile)	Mouse #1: 8 Mouse #2: 13 Mouse #3: 7 Mouse #4: 8	Mouse #1: 3 Mouse #2: 5 Mouse #3: 3 Mouse #4: 7 Mouse #5: 2
SRGAP2C (juvenile)	Mouse #1: 9 Mouse #2: 4 Mouse #3: 6 Mouse #4: 2 Mouse #5: 5 Mouse #6: 5	N/A
shSrgap2 + hSRGAP2A	Mouse #1: 10 Mouse #2: 3 Mouse #3: 3 Mouse #4: 6 Mouse #5: 5 Mouse #6: 4	Mouse #1: 5 Mouse #2: 4 Mouse #3: 3 Mouse #4: 3 Mouse #5: 4
shSrgap2 + EVH1dead	Mouse #1: 7 Mouse #2: 5 Mouse #3: 8 Mouse #4: 10	Mouse #1: 6 Mouse #2: 3 Mouse #3: 3 Mouse #4: 2 Mouse #5: 6 Mouse #6: 4
shSrgap2 + GAPdead	Mouse #1: 13 Mouse #2: 10 Mouse #3: 9	Mouse #1: 5 Mouse #2: 5 Mouse #3: 2 Mouse #4: 7 Mouse #5: 2 Mouse #6: 4
shSrgap2 + SH3dead	Mouse #1: 8 Mouse #2: 9 Mouse #3: 6 Mouse #4: 8	Mouse #1: 5 Mouse #2: 3 Mouse #3: 9 Mouse #4: 4 Mouse #5: 2
shControl (adult)	Mouse #1: 8 Mouse #2: 9 Mouse #3: 6 Mouse #4: 8	N/A
shSrgap2 (adult)	Mouse #1: 6 Mouse #2: 8 Mouse #3: 7	N/A
SRGAP2C (adult)	Mouse #1: 8 Mouse #2: 8 Mouse #3: 6	N/A

Table S1. Number of electroporated brains and dendrites analyzed per condition (related to Figures 1, 3, 4).
Table indicating the number of electroporated mice and the number of dendrites per animal analyzed in each condition (Mouse #identification number: number of dendrites analyzed). One dendrite per cell was analyzed. Dendritic spines and Gephyrin clusters were analyzed in different sets of neurons. Spines were quantified based on mVenus fluorescence. Gephyrin clusters were analyzed based on EGFP-GPHN fluorescence in neurons expressing soluble TdTomato to visualize neuronal morphology.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Constructs

EGFP-GPHN (clone P1) was obtained from H. Cline (TSRI, La Jolla, USA) and subcloned into pCIG2 (Hand et al., 2005) between Xma1 and Not1 by replacing the IRES-GFP fragment. pCI-SEP-GluA2 was obtained from Addgene (plasmid 24001) and subcloned by PCR into pCAG downstream of a CMV-enhancer/chicken-β-actin (CAG) promoter, by replacing EGFP between Xma1 and Not1. pCMV-SEP-γ2 $GABA_AR$ was a gift from A. Triller (IBENS, Paris, France). It was subloned by PCR into a lentiviral vector (gift from A. Maximov, TSRI, La Jolla, USA) 3' of a synapsin promoter, between Nhe1 and EcoR1. hSRGAP2A was tagged with 3xHA as described previously (Charrier et al., 2012). Indicated point mutations were introduced in hSRGAP2A and GPHN using the QuickChange mutagenesis kit (Agilent). All SRGAP2A mutants were HA-tagged and expressed under a CAG promoter. GFP-Homer1c and SRGAP2C-HA plasmids were described previously (Charrier et al., 2012).

shRNA constructs and validation

For *in utero* shRNA-mediated knock-down experiments, we used and modified the previously described pH1SCV2 vector (Charrier et al., 2012). In pH1SCV2, shRNA expression is driven by a H1 promoter. In addition, CAG promoter drives the expression of myristoylated-Venus (mVenus), allowing the identification of the electroporated cells and the visualization of their morphology. For co-expression of shRNAs with EGFP-GPHN, mVenus was replaced with the red fluorescent protein TdTomato between Xho1 and Not1. We named this vector pH1SCTdT2. Control shRNA (shControl) and shRNA against mouse *Srgap2a* (shSrgap2) were described previously (Charrier et al., 2012). ShGPHN used the seed sequence 5'-GCT ACA TCT CGC CTC TCT ACA-3' and was validated as described previously (Charrier et al., 2012). A GPHN mutant containing 4 silent mutations (t825a_a828g_t829a_c830g, named GPHN*) was designed to resist shGPHN-mediated knock-down. To knock-down mouse *Srgap2a* with lentivirus, we used a lentiviral vector expressing shRNA under a H1 promoter and TdTomato under a synapsin promoter. Lentiviral vectors were a gift from A. Maximov (TSRI, La Jolla, USA).

In utero cortical electroporation and slice preparation

In utero cortical electroporation was performed at E15.5 on timed pregnant F1 females from C57BL/6J (RRID:IMSR_JAX:000664) X 129/SvJ (RRID:IMSR_JAX:000691), timed pregnant RjOrl:SWISS females (Janvier Labs) for electrophysiology or C57BL/6J females Janvier Labs in Figure 5. The previously described protocol for *in utero* cortical electroporation (Hand and Polleux, 2011) was modified as follows. We used timed pregnant F1 females from C57BL/6 X 129/SvJ, except in electrophysiology and *in utero* gephyrin replacement experiments (Figure 5), where we used timed pregnant Swiss and C57BL/6 females, respectively. Endotoxin-free DNA was injected using a glass pipette into one ventricle of the mouse embryos. The volume of injected DNA was adjusted depending on the experiments. Electroporation was performed at E15.5 using a square wave electroporator (ECM 830, BTX) and gold paddles. The electroporation settings were: 4 pulses of 40 V for 50 ms with 500 ms interval. Plasmids were used at the following concentrations: shRNA vectors and reporter plasmids: 0.5 μg/μl (adults) or 1 μg/μl (juveniles); SRGAP2C-HA, hSRGAP2A-HA and hSRGAP2A-HA mutants: 1 μg/ml; EGFP-GPHN and SEP-γ2 $GABA_AR$: 0.3 μg/μl; SEP-GluA2 0.3 to 0.5 μg/μl; EGFP-GPHN* and EGFP-GPHN_PAPA*: 0.4 μg/μl. To visualize cortical neurons expressing SRGAP2C, SRGAP2C was co-electroporated with pH1SCV2 or pH1SCTdT2. Animals at the indicated age were anaesthetized with isofluorane before intracardiac perfusion with PBS and 4% paraformaldehyde (Electron Microscopy Sciences). Unless otherwise indicated, 100 μm coronal brain sections were obtained using a vibrating microtome (Leica VT1200S). Sections were mounted on slides in Vectashield. In case of SEP-γ2 $GABA_AR$ and SEP-GluA2 transfection, embryos were harvested at E18.5 in order to culture cortical neurons.

Lentivirus production

48 hours after HEK293 cell transfection, the viral supernatant was collected, centrifuged at 3,000 g for 5 min at 4ºC to remove cell debris, and ultracentrifuged at 25,000 × g for 2 hours on a 20% sucrose cushion. Pellets were resuspended in sterile PBS, aliquoted and stored at –80ºC.

Primary neuronal culture and lentiviral infection

Primary cultures were performed as described previously (Charrier et al., 2012) using timed pregnant C57BL/6J mice at E17.5-E18.5 with few modifications. Briefly, neurons were plated on glass coverslips coated with poly-D-

ornytine (80 μg/ml, Sigma) in MEM supplemented with sodium pyruvate, L-glutamine and 10% horse serum. Medium was changed 2-3 hours after plating with Neurobasal supplemented with L-glutamine (2 mM), B27 (1X) and penicillin (2.5 units/ml)-streptomycin (2.5 μg/ml). Then, one third of the medium was changed every 5-7 days. Unless otherwise indicated, all products were from Life Technologies. Cells were maintained at 37°C in 5% CO2 until use. When indicated, cortical neurons were infected 4 days after plating with concentrated lentiviruses driving the expression of shRNA and TdTomato. In these conditions, virtually all neurons were infected (data not shown).

Immunocytochemistry

Cells at the indicated age were fixed for 15 min at room temperature using 4% (w/v) paraformaldehyde in PBS, and incubated for 30 min in 0.3% Triton X100, 3% BSA (Sigma) in PBS to permeabilize and block nonspecific staining. Primary and secondary antibodies were diluted in the buffer described above. Primary antibodies were incubated for 1 hour at room temperature and secondary antibodies were incubated for 45 min at room temperature. Coverslips were mounted on slides with Vectashield (Vector Laboratories). Primary antibodies were mouse anti-Gephyrin (mAb7a, Synaptic System, 1:800, RRID:AB_2314591), mouse anti-Homer1 (clone 2G8, Synaptic System; 1:200, RRID:AB_2120992), mouse anti-PSD95 (clone K28/43, Neuromab, 1 :1,000, RRID:AB_2307331) and rabbit-anti-SRGAP2 C-terminal (raised against residues 873-890, 1:200, Guerrier et al., 2009, Charrier et al., 2012). All secondary antibodies were Alexa-conjugated (Invitrogen) and used at a 1:1,000 dilution.

Image acquisition and analysis on dissociated neurons

Fixed cell imaging. To analyze the association of Gephyrin and Homer with SRGAP2A, single section images were acquired using a spinning disk confocal microscope corresponding to a Yokogawa CSU10 scan head mounted on a Leica DM5000B microscope. Images were acquired using a 100X, NA 1.4 objective and collected using a COOLSNAP HQ2E CCD camera (Photometrics) controlled by Metamorph software (Molecular Devices). A procedure based on wavelet decomposition was used to detect, threshold and binarize fluorescent clusters. The fraction of Gephyrin and Homer1 clusters colocalized with SRGAP2A puncta was calculated using homemade software in MATLAB (MathWorks). This fraction was named "association index". Since SRGAP2 forms small puncta that are widely distributed, we used the mask of the n+1 SRGAP2 image to calculate the random fraction of Gephyrin or Homer clusters associated with SRGAP2A in the n image.

The effect of *Srgap2a* knock-down on Gephyrin, Homer1 and PSD95 were quantified on isolated cells immunoreactive to the protein of interest that were randomly chosen. Images were acquired in 12-bit mode using a DeltaVision widefield fluorescence imaging system (Applied Precision*)*, equipped with a 100X objective lens (NA 1.4), appropriate filters and a CCD camera. Exposure time was determined on bright control cells to avoid pixel saturation. All Gephyrin, Homer1 or PSD95 images from a given culture were then acquired with the same exposure time. Quantifications were performed blindly using the Nikon software NIS-Elements (Nikon Corporation, Melville, NY). A user-defined intensity threshold was applied to select clusters and avoid their coalescence. To calculate the normalized fluorescence intensity associated with endogenous Gephyrin, Homer1 or PSD95 clusters, the mean cluster fluorescence intensity was calculated for each dendrite (image). Each value was then expressed as a fraction of the average in control condition.

Live cell imaging. Live cell imaging was performed on a spinning disc confocal microscope corresponding to a Yokogawa CSUX1-A1 scan head mounted on a Nikon Eclipse Ti inverted stand. Excitation was provided by 491nm and 561nm lasers (Cobolt Calypso and Cobolt Jive, respectively). Images were acquired using a 100X Plan Apochromat, NA 1.4 objective or a 100X H-TIRF, NA 1.49 objective and collected using an Evolve EMCCD camera (Photometrics). The EMCCD Camera, piezo and laser line switching were controlled by Metamorph software (Molecular Devices). An environmental chamber (Life Imaging System) enclosing the microscope stand maintained the temperature at 37°C. After 21-22 days in culture, coverslips with electroporated cortical neurons were placed in a custom live cell imaging chamber. The recording medium contained (in mM): 130 NaCl, 5 KCl, 2 $CaCl_2$, 10 HEPES, 30 glucose, and 1 $MgCl_2$, (pH 7.4). Z-stacks of images were acquired with spacing of 100 nm. Images were blindly analyzed using the Nikon software NIS-Elements (Nikon Corporation, Melville, NY). Briefly, images were filtered for peak intensity to enhance cluster outlines, subjected to z-stack maximum projection and fluorescence intensity was manually thresholded. For each image, the threshold was adjusted on the brightest clusters. This processing allowed to clearly discriminate receptor clusters from diffuse fluorescence but likely results in an underestimation of small clusters.

Confocal image acquisition and analysis on brain slices

Confocal images of electroporated neurons in slices were acquired in 1024x1024 mode using an A1R laser scanning

confocal microscope controlled by the Nikon software NIS-Elements (Nikon Corporation, Melville, NY) or a Leica TCS SP8 confocal laser scanning platform controlled by the LAF AS software and equipped with a tunable white laser and hybrid detector (Leica Microsystems). We used the following objective lenses: 10X PlanApo, NA 0.45 (identification of electroporated neurons and low magnification images) and 100X H-TIRF, NA 1.49 (Nikon) or 100X HC-PL APO, NA 1.44 CORR CS (Leica) (images of spines and Gephyrin clusters). Images were blindly acquired and analyzed. Z-stacks of images were acquired with spacing of 150 nm. Dendritic spines and Gephyrin clusters were analyzed in different sets of neurons. Spines were quantified based on mVenus fluorescence conveyed by the shRNA vector (pH1SCV2). Gephyrin clusters were quantified based on EGFP-GPHN fluorescence and soluble TdTomato driven by the shRNA vector (pH1SCTdT2). All quantifications were performed in the somatosensory cortex, in sections of comparable rostro-caudal position. Gephyrin clusters and dendritic spines were quantified in the proximal part of oblique dendrites directly originating from the apical trunk. Only dendrites that were largely parallel to the plane of the slice were analyzed (usually no more than 1 dendrite per neuron). The morphometric analysis of dendritic spines has been described previously (Charrier et al., 2012). For Gephyrin clusters, the equivalent diameter (d) was defined as follows: $d = \sqrt{(a \times b)}$, were a is the major axis and b is the minor axis. The subcellular localization of EGFP-Gephyrin clusters (spine or shaft) was determined by comparison with the image of TdTomato expressed in the electroporated neurons to visualize their morphology. The density of Gephyrin clusters along dendrites was calculated as described previously for dendritic spines (Charrier et al., 2012). Gephyrin clusters were quantified over an average of 60 μm of dendrite. The length of the dendritic segment was measured on the z projection.

Electrophysiology

Acute coronal brain slices (300 μm thick) were obtained from juvenile (postnatal day 20–25) Swiss mice electroporated *in utero* with shSrgap2 in pH1SCTdT2. Whole-cell patch-clamp recordings were performed in layer 2/3 cortical pyramidal neurons of the somatosensory cortex. Briefly, after decapitation the brain was quickly removed from the skull and placed in ice-cold (4°C) 'cutting solution' containing (in mM): 200 sucrose, 25 glucose, 3 KCl, 1.25 NaH2PO4, 26 NaHCO3, 0,8 CaCl2, and 8 MgCl2, saturated with 95% O2 and 5% CO2 (pH 7.3–7.4). Slices were cut using the 7000 smz tissue slicer (Campden Instrument). Slices recovered in oxygenated artificial cerebrospinal fluid (ACSF) containing (in mM): 125 NaCl, 2.5 KCl, 2 CaCl2, 1 MgSO4, 1.25 NaH2PO4, 26 NaHCO3, and 16 glucose (pH 7.4), at 36°C for 30 minutes and then at room temperature for at least 45 minutes. For electrophysiological recordings, slice were transferred to a submerged recording chamber and continuously perfused at 33–34°C with oxygenated ACSF at a rate of 5-6ml/min. Inhibitory and excitatory miniature post-synaptic miniature currents (mIPSCs and mEPSCs, respectively) were recorded at a holding potential of −60 mV in the presence of 0.5 μM TTX. mIPSCs were isolated by adding NBQX (10 μM) and D-AP5 (50 μM) to the ACSF. mEPSCs were isolated using gabazine (10 μM). Glutamatergic currents were recorded using an intracellular solution containing (in mM): 144 K-gluconate, 7 KCl, 10 HEPES, 10 EGTA, 4 MgATP, 0.6 NaGTP, (pH adjusted to ~7.3 with KOH). For GABAergic currents an intracellular solution containing (in mM): CsCl 140, Hepes 10, BAPTA 1, MgATP 4, MgCl2 1, CaCl2 1, 10 EGTA and 5 QX-314 (pH adjusted to ~7.3 with CsOH) was used. Access and input resistance were monitored by applying 5 mV hyperpolarizing steps of current. Cells showing > 20% change in these parameters were excluded from the analysis. Data were sampled at 10 kHz and filtered at 2-3 kHz. mIPSCs and mEPSCs were analyzed over 1-3 min periods using pClamp 10.0 (Molecular Devices). Overlapping events were excluded from amplitude analysis. All drugs were obtained from Tocris Cookson or Latoxan.

Cell line culture, transfection and lysis

HEK293 (CRL-1573, RRID:CVCL_0045) cell line was obtained from ATCC and cultured according to suggested protocols. Transfection was performed using Jet-Prime (Polyplus Transfection) according to the manufacturer protocol. Cells were collected 48 hours after transfection. Cells were lysed using NP-40 buffer (1% NP-40, 150 mM sodium chloride, 50 mM Tris buffer at pH 7.4) under agitation for 1 hour at 4ºC.

Subcellular fractionation

Subcellular fractionation was performed from P15 mouse brains as described in (Perez-Otano et al., 2006) with minor modifications. All steps were performed at 4ºC. Briefly, brains were homogenized in ice-cold HEPES-buffered sucrose (0.32 M sucrose, 4 mM HEPES pH 7.4, 2 mM EDTA, protease inhibitor cocktail), phosphatase inhibitor coktail (Sigma)) using a motor driven glass-teflon homogenizer. The homogenate was centrifuged at 800 g for 10 min. The resulting supernatant was centrifuged at 10,000 g for 15 min, yielding the crude synaptosomal pellet. The pellet was resuspended in sucrose buffer and centrifuged one more time at 10,000 g for 15 min. The crude

synaptosome fraction was then subjected to hypo-osmotic shock and centrifuged at 25,000 g for 20 min. The resulting pellet was lysed for 1 hour using sucrose buffer supplemented with 1% CHAPS (Sigma) and centrifuged at 10,000 g for 10 min. The corresponding supernatant is referred to as synaptic fraction or synaptic membranes. Protein concentration was measured and protein samples were prepared for Western blotting or co-immunoprecipitation. To assess the relative levels of proteins in synaptic fractions, 2-3 brains of wild-type or knock-out mice from the same litter were pooled. Four litters were used for quantitative analysis.

Co-immunoprecipitation

For HEK cells, 1 mg of total protein from each sample was diluted in NP-40 buffer and incubated overnight at 4°C, with either 5 µg of mouse anti-HA antibody (HA.11 Clone 16B12 Monoclonal Antibody, Covance) or 5 µg of mouse IgG as negative control. For Homer1 co-immunoprecipitation in brain extracts, 2 mg of total protein were diluted in Sucrose buffer supplemented with 1% CHAPS, and incubated overnight at 4°C, with either 9 µg of mouse anti-Homer antibody (Synaptic system) or equivalent amount of mouse IgG. Protein G-agarose beads (Roche) were then added for 2-3 hours. After extensive washes, the beads were resuspended in gel-loading buffer and bound proteins were released with boiling. Inputs correspond to 20-25 µg of proteins. For Gephyrin co-immunoprecipitation in brain extracts, 2 mg of total protein were diluted in a Tris-NaCl buffer containing (50mM Tris pH=7.4, 150mM NaCl, 2mM EDTA, protease inhibitor cocktail, phosphatase cocktail inhibitors, Sigma) supplemented with 0.1% CHAPS and incubated overnight at 4°C with 25 µl of purified GPHN.FingR, or nothing in control condition. Anti-Flag M2-coupled magnetic beads (Sigma) were then added for 2 hours. The beads were washed 6 times using a buffer containing 50 mM Tris pH=7.4, 200 mM NaCl, 2mM EDTA, 0.1% CHAPS, protease inhibitor cocktail, phosphatase inhibitor cocktail. Bound proteins were released with boiling in gel-loading buffer. Input corresponds to 50 µg of proteins. Each experiment was reproduced at least three times.

Western blotting and Antibodies

Proteins were separated by SDS-PAGE and transferred to a 0.45 µm polyvinylidene difluoride (PVDF) membrane (Amersham). Western blotting was performed using the following primary antibodies: mouse anti-HA.11 (clone 16B12, Covance, 1:2,000, RRID:AB_291259), rabbit anti-GFP (Life Technologies, 1:2,000, RRID:AB_10073917), mouse anti-actin (clone C4, Millipore, 1:5,000, RRID:AB_2223041), rabbit-anti-SRGAP2 C-terminal (raised against residues 873-890, 1:4,000, Guerrier et al., 2009), rabbit-anti-SRGAP2 N-terminal (raised against residues 193-205, 1:500, Charrier et al., 2012), mouse anti-Homer1 (clone 2G8, Synaptic System; 1:2,000, RRID:AB_2120992), mouse anti-PSD95 (clone K28/43, Neuromab, 1 :2,000, RRID:AB_2307331), mouse anti-GluN2B (clone N59/20, Neuromab, 1 :500, RRID:AB_10673405), rabbit anti-GluN2A (Millipore, 1 :1,000, RRID:AB_310837), mouse anti-GluA2 (BD Biosciences, 1 :200, RRID:AB_396373), rabbit anti-GluA1 (Millipore, 1:500, RRID:AB_2113602), mouse anti-Gephyrin (clone G-6, Santa Cruz, 1:500, RRID:AB_627670), rabbit anti-GAPDH (Synaptic System, 1:2,000, RRID:AB_10804053) and HRP-conjugated secondary antibodies. Protein visualization was performed by chemiluminescence using a Fluorochem Q imager (ProteinSimple). When needed, signal intensity was quantified using ImageJ. The signal associated with the protein of interest was normalized to signal associated with actin in the same lane. We then compared the relative signals in wild-type and knock-out conditions.

Production and purification of GPHN.FingR

pCAG_GPHN.FingR-eGFP-CCR5TC (Addgene, plasmid #46296) was modified as follow to allow production and purification of the GPHN.FingR at the protein level. Briefly, the GPHN.FingR gene was subcloned by PCR into pET-20b+ vector between SacI and XhoI. The cloning procedure conserved the EGFP and Flag tags but added a pelB signal peptide in N-terminus and a His-tag at the extreme C-terminus. This His-tagged GPHN.FingR protein was produced in BL21 Star (DE3) bacteria. Expression was induced using 1 mM IPTG in LB medium overnight at 20°C. Bacteria were pelleted at 5,000 g for 30 min at 4°C and lysed using One Shot Cell Disrupter continuous system in lysis buffer (20 mM Tris pH 8, 500 mM NaCl, 10 mM Imidazole, 0.1% CHAPS and protease inhibitor cocktail). The lysate was centrifuged at 40,000 rpm for 40 minutes at 4°C with a 70Ti rotor (Beckman Coulter). The supernatant was loaded on a 10 ml affinity column resin (Ni Sepharose 6 Fast-Flow, GE Healthcare). After extensive washing, GPHN.FingR was eluted with 100 mM Imidazole (20 mM Tris pH 8, 200 mM NaCl), aliquoted and frozen with 20% glycerol for storage at -20°C.

Statistics

Data are a minimum of three independent experiments unless stated otherwise. For dendritic spine and Gephyrin cluster analysis, all data were obtained from at least three independent experiments or at least three animals from two

independent litters. Statistical analyses were performed with Prism (GraphPad Software). For normalization, the density or the fluorescence intensity in individual neurons was normalized to the average value in control condition. For statistical analysis, we first checked the normality of the distributions using the one-sample Kolmogorov-Smirnov test. We used non-parametric tests (Mann-Whitney test or the Kruskal-Wallis test followed by the Dunn's Multiple comparison test) when at least one of the group tested displayed a distribution that deviated significantly from normality. A test was considered significant when $p < 0.05$. Data represent the distribution (or the mean) of the mean value per cell in the main figures and the distribution of individual spines or clusters in Figures S2 and S4. See also table S1.