SUPPLEMENTARY MATERIAL AND METHODS

Antibodies

The antibody α17141 was obtained by immunizing rabbits with GST fused to amino acids 112 to 305 of the SCHIP1a isoform (amino acids 187 to 380 of the IQCJ-SCHIP1 isoform; supplementary material Fig. S2A) (Goutebroze et al., 2000). The antibody α16280 was obtained by immunizing rabbits with GST fused to the amino acids encoded by *Schip1* exon 6 (amino acids 98 to 325 of IQCJ-SCHIP1; supplementary material Fig. S2A). The antibody HPA was from Sigma (#HPA003445) and was obtained by immunizing rabbits with a region of SCHIP1a encompassing amino-acids 47 to 165 (amino acids 122 to 240 of IQCJ-SCHIP1; supplementary material Fig. S2A). The antibody α959 was obtained by immunizing chickens with GST fused to a protein encompassing the last C-terminal 179 amino acids of SCHIP1 common to all isoforms (amino acids 381 to 559 of IQCJ-SCHIP1: supplementary material Fig. S2A) (Goutebroze et al., 2000). These antibodies where characterized by immunoblotting on lysates from COS-7 cells transfected with expressing vectors for Flag-tagged SCHIP1 isoforms (pFLAG-CMV-2 vector, Sigma-Aldrich) or Flag-tagged IQCJ-SCHIP1 truncated proteins corresponding to the cDNA of IQCJ-SCHIP1 deleted of exon 10, exons 10 and 11, or exons 10 to 12. The antibodies α17141, α16280 and αHPA recognized specifically the SCHIP1a and IQCJ-SCHIP1 isoforms, but not the four other SCHIP1 isoforms, indicating that the immunoglobulins are directed against the region encoded by *Schip1* exon 6, which is not present in the four other SCHIP1 isoforms (supplementary material Fig. S2B). They also recognized IQCJ-SCHIP1 truncated proteins (supplementary material Fig. S2C). The antibody α959 recognized all SCHIP1 isoforms but not truncated proteins (supplementary material Fig. S2B,C).

Identification of mouse SCHIP1 isoforms, and isoform mRNA expression analysis

The sequences coding for the mouse SCHIP1 isoforms SCHIP1a, SCHIP1b and IQCJ-SCHIP1, were previously identified (Martin et al., 2008) (GenBank accession numbers: SCHIP1a, EU163407; SCHIP1b, EU163408; IQCJs-SCHIP1, EU163409). The sequences coding for isoforms SCHIP1c, SCHIP1d and IQCJs-SCHIP1, were identified by screening the mouse genome and EST databases at NCBI using the basic local alignment search tool. The corresponding cDNAs were isolated by reverse transcription/amplification (RT/PCR) experiments from mouse brain mRNA and sequenced. The cDNA sequences were deposited in GenBank (accession numbers: SCHIP1c, KM233716; SCHIP1d, KM233715; IQCJs-SCHIP1, KJ941154). For isoform mRNA expression analysis, RT/PCR were performed using a common antisense primer (5'- CGGGACAGTAAGCTCGGACGTTAC) and the following isoform specific sense primers:

SCHIP1a (5'- GCAAGCTGACAGAGATTACCGAGAGG);

SCHIP1b (5'- GCAAGCTGACAGAGCACAGAAGAATGAG);

SCHIP1c (5'- TCAGGAGAACTGCTCGTACCAGG);

SCHIP1d (5'- GCAGTTGGCACTTCAGCCTGTCA);

IQCJ-SCHIP1 (5'- CAGCAGCACACCCGATTACCGAGAGG);

IQCJs-SCHIP1 (5'- CAGCAGCACACCCGCACAGAAGAATGAG).

The expected sizes of amplified DNA fragments were the following: SCHIP1a, 1549 bp; SCHIP1b, 853 bp; SCHIP1c, 865 bp; SCHIP1d, 939 bp; IQCJ-SCHIP1, 1537 bp; IQCJs-SCHIP1, 853 bp.

For expression analysis of the housekeeping gene peptidylpropyl isomerase B (Cyclophilin B, Cyp), RT/PCR was performed using the primers 5'- CCATCGTGTCATCAAGGACTT and 5'- TTGCCATCCAGCCAGGAGGTC. The expected size of the amplified DNA was 215 bp.

Generation of *Schip1*Δ10 mutant mice

Schip1 mutant mice were generated by deletion of exon 10 of *Schip1*, using a three-lox recombination strategy.

Generation of Schip1$^{flox10/+}$ ES cells

A DNA fragment containing *Schip1* exon 10 surrounded by 3.6 kb endogenous DNA upstream and 5.6 kb endogenous DNA downstream was PCR-amplified in two steps and inserted (using PCR-added *Nsi*I and *Sal*I restriction sites) in a pBR322 vector containing a *NsiI-Sal*I polylinker from pBluescript KS II (Stratagene). A *lox*P sequence was added upstream from exon 10 by PCR. Next, a 2 kb *XmaI-XmaI* fragment containing a floxed PGK*Hygromycin*/*GFP* cassette was inserted 0.8 kb downstream of exon 10 (in the same orientation as the *Schip1* gene).

The *Nsi*I-*Sal1 Schip1-lox10* targeting fragment was electroporated into ES cells (line E14 subclone IB10 (129/Ola)) (Robanus-Maandag et al., 1998) using a Bio-Rad Gene Pulser (0.8kV, 1µF, discharge 0.1 ms, 0.4 cm electrode distance, cells in 200 µl PBS). Electroporated cells were plated on 6 x 60 cm^2 mouse embryonic fibroblasts. After 24 h, hygromycin B 150 µg/ml (Calbiochem) was added for 3 days. Hygromycin-resistant cells were trypsinized and GFP-expressing cells were isolated by flow cytometric analysis and plated on 5 x 96-well microplates. Seven homologous recombinants out of 77 selected clones were identified by long-range PCR analysis. The caryotype of these clones was analyzed. The genomic DNAs were extracted for sequencing and Southern blots were performed to verify 5' and 3' homologous recombination with a probe corresponding to the PGK*Hygromycin*/*GFP* cassette. One clone was then chosen for injection.

Generation and genotyping of Schip1mutant mice

Germline chimeras (*Schip1$^{flox10HygGFP/+}$*) were generated by injection of 10 *Schip1* mutant ES cells into blastocysts and crossed with C57BL/6 mice to produce outbred heterozygous

offspring. The genotypes of all offspring were analyzed by PCR of tail-tip DNA. To generate *Schip1*$^{\Delta 10/+}$ mice, *Schip1*$^{flox10HygGFP/+}$ mice were crossed with *MeuCre* deletor mice (Leneuve et al., 2003). In the derived double transgenic offspring tail DNA the *Schip1*$^{\Delta 10}$ allele was detected by PCR with primers C (5'- CAGACAGCAGACTATCATGGGG) and D (5'- AATGACTGTTCTGAGCACGG) amplifying a 551-bp product and the *Schip1*$^{+}$ allele was detected with primers A (5'- GGGTCACTAAGTTCTCCACATGA) and B (5'- TTGACCACTGAGCCATCTCTGCA) amplifying a 401-bp product. Mosaic mice carrying the *Schip1*$^{\Delta 10}$ allele were subsequently crossed with C57BL/6J mice to segregate the mutant allele and obtain *Schip1*$^{\Delta 10/+}$ mice. Mutant mice were then backcrossed on a C57BL/6J background for at least 10 generations before experiments described here.

Schip1$^{Gt(ROSA)77Sor}$ mice

129S-*Schip1*$^{Gt(ROSA)77Sor}$/J mutant mice (129sv genetic background) were produced by the laboratory of P. Soriano (Chen et al., 2004). They were obtained from the Jackson Laboratory and backcrossed on a C57BL/6J background for at least 10 generations before experiments described here (*Schip1*$^{Gt(ROSA)77Sor}$ or Gt mice). In these mice, the retroviral gene-trap vector ROSAFARY was targeted to intron 5 of *Schip1* (position of the gene-trap defined according to our characterization of the *Schip1* gene). Briefly, this gene-trap vector was designed with a promotor trap module, a *frt*-flanked poly-A trap module, and a promoterless *lacZ-neo* reporter fusion gene, which functions as an artificial 3' terminal exon to intercept and terminate transcription from the targeted promoter. After inserting in an intron of an endogenous gene at a permissive site and in the correct orientation, the promoter trap module and the poly-A trap module can be activated to form fusion transcripts with the 5' or 3' exons, respectively.

Animal procedures

Research was conducted according to national and international guidelines (EC directive 86/609). The laboratory was approved to carry out animal experiments by the *Direction Départementale des Services Vétérinaires de Paris, Service de la Protection et de la Santé Animales et de la Protection de l'Environnement* (licence B75-05-22). The principal investigator had a personal authorization (L Goutebroze, licence 75-1533). Mice were group-housed with *ad libitum* access to food and water and a 12 hour - 12 hour light - dark cycle (light phase onset at 7 a.m.). For staging of embryos, the day of vaginal plug was considered E0.5.

Immunoprecipitations from brain lysates

For preparation of brain extracts, brains were homogenized in a buffer containing 50 mM Tris pH 7.8, 3 mM $MgCl_2$, 320 mM sucrose and Complete protease inhibitors (Roche), using a Heidolph homogenizer. The homogenates were centrifuged 15 min at 4°C at 1000 g. The supernatants were centrifuged at 100 000 g at 4°C for 1 h and the pellets were extracted for 30 min on ice in a buffer containing 10 mM Tris, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate and Complete protease inhibitors (Roche). The detergent-insoluble material was removed by centrifugation at 100 000 g for 1 h at 4°C. The protein A beads, preincubated with the respective antibody α17141 or HPA, were added to the supernatants and incubated overnight at 4 °C with rotation. Beads were washed with a buffer containing 20 mM Tris pH 7.6, 150 mM NaCl, 0.5% Triton X-100 and Complete protease inhibitors (Roche), precipitated proteins were then loaded on NuPAGE Bis-Tris gels (Invitrogen) and transferred to 0.45 µm Nitrocellulose membranes. Membranes were blocked with TBST buffer (5% dry milk in TBS - 0.1% Tween), 1 h at room temperature, incubated overnight at 4°C with the rabbit antibody α16280 or the chicken antibody α959, and then 1 h

with IRDye^{TM}800CW or IRDye^{TM}680CW secondary antibodies, before development using Odyssey Imaging System (LI-COR Biosciences).

Tissue processing

Adult mice (2 to 3-month old) and E14.5 embryos (for cleaved caspase 3 immunostaining) were deeply anesthetized with pentobarbital (500 mg/kg i.p., Sanofi-Aventis, France) and transcardially perfused with 4% paraformaldehyde (PFA) in 0.12 M phosphate buffer (PB) pH 7.4 for 10 min. E13.5, E14.5, E16.5, E18.5 embryos and P0 newborn mice were rapidly decapitated. Brains (adult and P0 mice; E16.5 and E18.5 embryos) and heads (E13.5 and E14.5 embryos) were fixed or postfixed in 4% PFA overnight at 4°C and cryoprotected in a 30% sucrose/sodium phosphate buffered saline (PBS) solution for 24 h at 4°C. Five hundred µm-thick horizontal sections of adult brains were produced using a vibratome (Leica, France) for phase contrast analysis. For NF160 immunostaining and X-gal staining, 30 µm-thick coronal sections of adult brains were produced using a cryotome (microm KS-34, Thermo Scientific, France) and kept at -20°C in cryoprotectant solution (30% ethylene glycol/30% glycerol/0.12 M PB) until use. For MOG immunostaining and AChE staining, 30 µm-thick sagittal and 40 µm-thick coronal sections of adult brains were produced using a vibratome (Leica, France) and kept at -20°C in the cryoprotectant solution. Brains and heads of P0 mice and embryos were embedded in 7.5% gelatin, 10% sucrose, and 0.12 M PB, snap frozen in isopentane and stored at -20°C until sectioning. Coronal and horizontal 20 µm-thick sections were produced using a cryostat (Microm Microtech, France), thaw mounted onto Superfrost Plus microscope slides (Thermo Fischer Scientific), air-dried and stored at -20°C until use.

Primary cultures of dissociated neurons

The piriform cortex from E14.5 embryos was dissected in ice-cold 0.02 M HEPES in Ca^{2+} /Mg^{2+}-free HBSS (Sigma), and mechanically dissociated after trypsin (2.5 mg/ml) incubation. Dissociated cells were plated on 14 mm-diameter coverslips ($0.3x10^5$ cells/coverslip) coated with poly-L-lysine (0.25 mg/ml) (Sigma, #P7890) and natural mouse laminin (0.01 mg/ml) (Invitrogen, #23017015), and cultured in Neurobasal medium supplemented with B27 (Gibco®) serum-free supplement, Glutamax (Invitrogen), and 1% penicillin/streptomycin at 37°C in presence of 5% CO_2. Neurons were fixed for immunostaining by adding in the medium ½ volume of preheated 8% PFA/20% sucrose in PBS for 20 minutes. For time-lapse imaging, neurons were grown in µ-Slide 8 well-ibiTreat Microscopy Chambers (Ibidi, #80826) at $0.3x10^5$/well coated as described above.

Immunostaining

Fixed neurons/explants on coverslips or tissue sections were incubated in permeabilization/saturation solution PS (0.25% Triton X-100, 5% bovine serum albumin, PBS) or PGT (0.25% Triton X-100, 0.2% gelatin, PBS) for 2 h at room temperature (RT) and then incubated with primary antibodies diluted in the same solution overnight at 4°C. After 3 PBS washes, incubation with Alexa Fluor conjugated-secondary antibodies diluted in PS or PGT solution was performed for 2 h at RT. Then, coverslips and tissue sections were washed again 3 times in PBS and mounted with DAPI-containing Vectashield (Vector Lab). For membrane-bound EPHB2-Fc immunolabeling, fixed neurons were not permeabilized and all incubations were performed in 5% bovine serum albumin/PBS. EPHB2-Fc was detected using a human anti-IgG1 antibody. To visualize F-actin, neurons were incubated for 30 min with Alexa Fluor 488/546/633 conjugated-phalloidin (Molecular Probes) diluted in PBS after secondary antibody incubation.

Acetylcholinesterase staining

Sections were incubated in staining solution (1.7 mM acethylthiocholine iodide, 3 mM copper sulphate, 10 mM sodium citrate, 0.5 mM potassium ferricyanide, 0.2 mM ethopropazine, 0.05 M sodium acetate buffer pH 5.5), rinsed in 0.05 M sodium acetate buffer pH 5.5, deshydrated and mounted with Eukitt® quick-hardening mounting medium (Sigma, #03989).

Nissl coloration

E14.5, E16.5, P0 and adult cryostat coronal sections were treated in sequential baths: 1X 70% ethanol (EtOH), 1X 95% EtOH, 2X 100% EtOH, 1X 95% EtOH, 1X 70% EtOH, 10 seconds/bath. Sections were then stained with cresyl violet (5 mg/ml in 0.6% acetic acid) for 2 minutes, and post-treated in 1X 70% EtOH, 1X 95% EtOH, 2X 100% EtOH, 2X xylene, 10 seconds/bath. Sections were finally mounted with Eukitt® quick-hardening mounting medium.

X-gal staining

Sections were incubated in staining solution (5 mM ferricyanide, 5 mM ferrocyanide, 2 mM $MgCl_2$, 0.1% Triton X-100, 1 mg/ml X-gal, PBS) for 2 h at room temperature, rinsed in PBS, mounted on slides and air-dried overnight. Sections were then incubated in H_20 for 3 minutes, stained in a 0.01% safranine solution, rinsed in H_20 and treated in sequential baths: 1X 70% EtOH, 1X 96% EtOH, 1X 100% EtOH, 2X xylene, 20 seconds/bath. Sections were finally mounted with Eukitt® quick-hardening mounting medium.

BrdU assay

Timed-pregnant heterozygous females at E12.5 received an intraperitoneal injection of 50 mg/kg BrdU (5-bromo-2-deoxyuridine, Sigma). E14.5 or E18.5 embryos were then collected

and tissue prepared as described above. For BrdU immunostaining, sections were permeabilized and blocked in 0.2% Triton X-100/4% BSA/PBS for 2 h at RT. DNA was denaturated with pre-heated 2 N HCl for 40 min at 37°C and sections were then washed in PBS. The following procedure was identical to that described above for immunostaining.

Image acquisitions

Immunolabeled and Nissl stained brain sections (excepted for MOG immunolabeling) were scanned with a Hamamatsu Nanozoomer Digital Pathology 2.0 HT (High Throughput) device (Hamamatsu Photonics, Japan and NDPView Nanozoomer associated software), with fluorescence unit option (L11600-05), and the NanoZoomer's 3-CCD TDI (Time Delay Integration) camera (Hamamatsu Photonics), resolution of 0.23 µm/pixel (40X). MOG-immunolabeled and X-gal-stained sections were imaged with a macroscope MVX10 (Olympus). AChE stained sections were imaged with a Provis light microscope (Olympus). BrdU stained brain sections, immunolabeled neurons and explants were imaged using an epifluorescence DM6000-2 Leica microscope.

For time-lapse imaging, neurons were placed on a motorized stage and maintained at 37°C using a thermostated chamber. Transilluminated time-lapse microscopy was performed using an inverted microscope DMI4000 Leica with a 63x/1.4 N.A. objective (1 acquisition/20 sec during 15 min, z=5 µm, z-step=1 µm).

Visualization of AC axons by fluorescent lipophilic tracer

1,1'-Dioctadecyl-3,3,3',3'-Tetramethylindocarbocyanine Perchlorate ('DiI'; DiIC18(3), Invitrogen, #D-3911) crystals were used to visualize the pathway of AC axons. Brains from E16.5 embryos were fixed for one week in 4% PFA/0.12 M PB. DiI crystals were injected at the base of the olfactory bulb to target the AON/anterior piriform cortex or in the posterior

piriform cortex. Brains were then incubated in PBS for 15 days at 37°C, embedded in 3% agarose/PBS, sliced into 100 µm-thick horizontal sections with vibratome (Leica, France), and imaged with a macroscope MVX10 (Olympus).

In situ hybridization

E13.5, E14.5 embryonic and P0 mouse brains were fixed by immersion with 4% PFA in PBS. Brains were postfixed for 2 h, cryoprotected in 10% sucrose/PBS, frozen in isopentane and stored at -80°C. Serial sections (20 µm) were cut with a cryostat (Microm Microtech, France) and stored at -80°C before hybridization. *Schip1* sense and antisense riboprobes were generated and labeled with digoxigenin-d-UTP by *in vitro* transcription (Roche Diagnostics) from a mouse *Schip1* cDNA encoding the C-terminal residues conserved in all SCHIP1 isoforms (nt[975-1758], GenBank accession number EU163407) cloned in pBluescript II KS+. Antisense riboprobes for guidance molecules were generated by *in vitro* transcription from vectors previously described : NPN2 (Chen et al., 1997); SEMA3F (Falk et al., 2005), generous gift from Pr. A Püschel; EPHB2 (*sek-3*) (Becker et al., 1994). *In situ* hybridizations were performed on tissue sections as described previously (Moreau-Fauvarque et al., 2003). Brain sections were scanned with the Hamamatsu Nanozoomer Digital Pathology (NDP) device as described above.

Statistical analysis

Statistical analyses were performed with StatView software, Abacus. For brain analyses along the antero-posterior axis or during development normally distributed data were subjected to factorial two-way ANOVA with genotype and brain region or age as between-group factors. Significant main effects were further analyzed by post hoc comparisons of means using the Newman–Keuls test. For the other analyses, variables that followed a normal distribution

were subjected to the Student t-test. For variables that did not follow a normal distribution, statistical analyses were carried out using the Mann–Whitney rank sum test to compare quantitative variables. Proportions were compared using the Chi² test. The significance was established at a P-value < 0.05.

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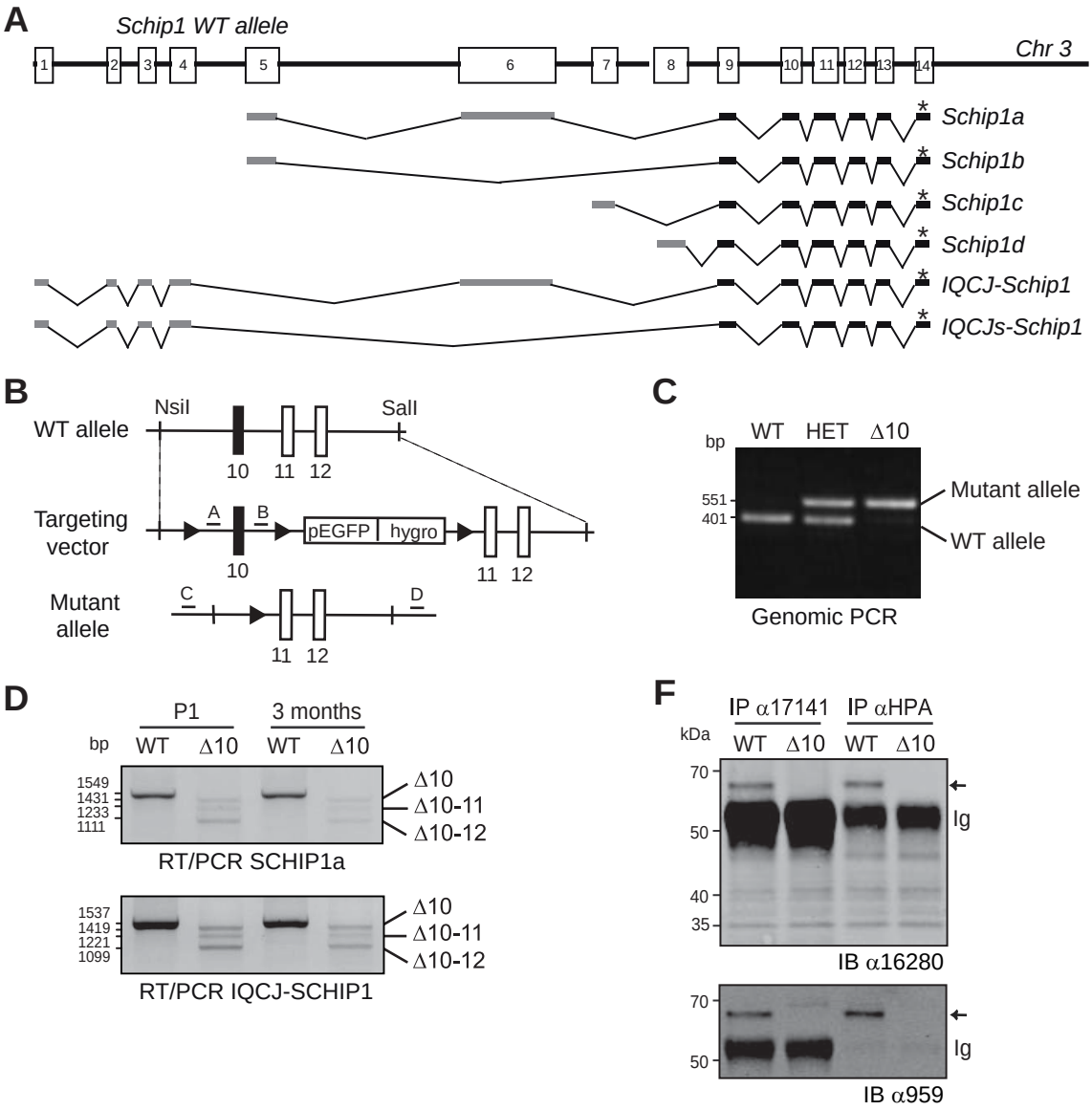

Fig. S1. Generation and characterization of *Schip1* mutant mice.

(A) Schematic representation of mouse *Schip1* isoforms. The *Schip1* gene is composed of 14 exons localized on mouse chromosome 3 and encodes six isoforms expressed in mouse brain. All isoforms differ in their N-terminus and share a C-terminal domain encoded by exons 9 to 14. Black boxes, exons common to all isoforms. Grey boxes, exons specific for one or two isoforms only. *, stop codon. (B) Schematic representation of *Schip1* gene-targeting three-lox strategy. Upper panel, *Schip1* genomic locus including exons 10, 11 and 12. Middle panel,

targeting vector with the three *lox*P sites (black arrowheads) flanking exon 10 and the selection cassette PGK*Hygromycin*/*GFP* (pEGFP/hygro). Lower panel, *Schip1* mutant allele obtained after *in vivo* Cre recombination. Positions of primers used for genotyping (A-D) are indicated. (C) PCR analysis on genomic DNAs of WT, heterozygous (HET) and homozygous *Schip1*Δ10 (Δ10). (D) RT-PCR analysis of SCHIP1a and IQCJ-SCHIP1 mRNAs from WT and Δ10 brains at post-natal day 1 (P1) and 3 months revealing, in mutant brains, low levels of DNA fragments that correspond to cDNAs deleted for the nucleic acids from exon 10 (Δ10) as expected, but also for the nucleic acids from exons 10 and 11 (Δ10-11), and from exons 10, 11 and 12 (Δ10-11-12). (E) Predicted C-terminal amino acid sequences of SCHIP1 mutant proteins. Deletion of exon 10 results in a frameshift, which creates a stop codon in exon 11, leading to the expression of mutant proteins, if any, lacking an 194-residue C-terminal part common to all SCHIP1 isoforms and with a novel unrelated 50-residue extension. Deletions of exons 10-11 and exons 10 to 12 also result in frameshifts, which create stop codons in exons 12 and 13, respectively. Dashed lines substitute for 147 amino acids of WT SCHIP1 proteins. Amino acids encoded by exon 10 are indicated in grey. C-terminal unrelated amino acids encoded by mutant cDNAs Δ10, Δ10-11 and Δ10-12, are indicated in grey and italics. (F) SCHIP1 expression in brain of WT and Δ10 mice. Immunoprecipitations (IP) performed with two different SCHIP1 antibodies, α17141 and HPA, followed by immunoblotting (IB) with two other antibodies α16280 and α959, reveal a protein at the expected molecular weight (65 kDa) for IQCJ-SCHIP1 in WT brain extracts (arrow), which is not detected in Δ10 brain extracts. C-terminal truncated proteins were not detectable in brain extracts from mutant mice (IB α16280). Ig, immunoglobulins.

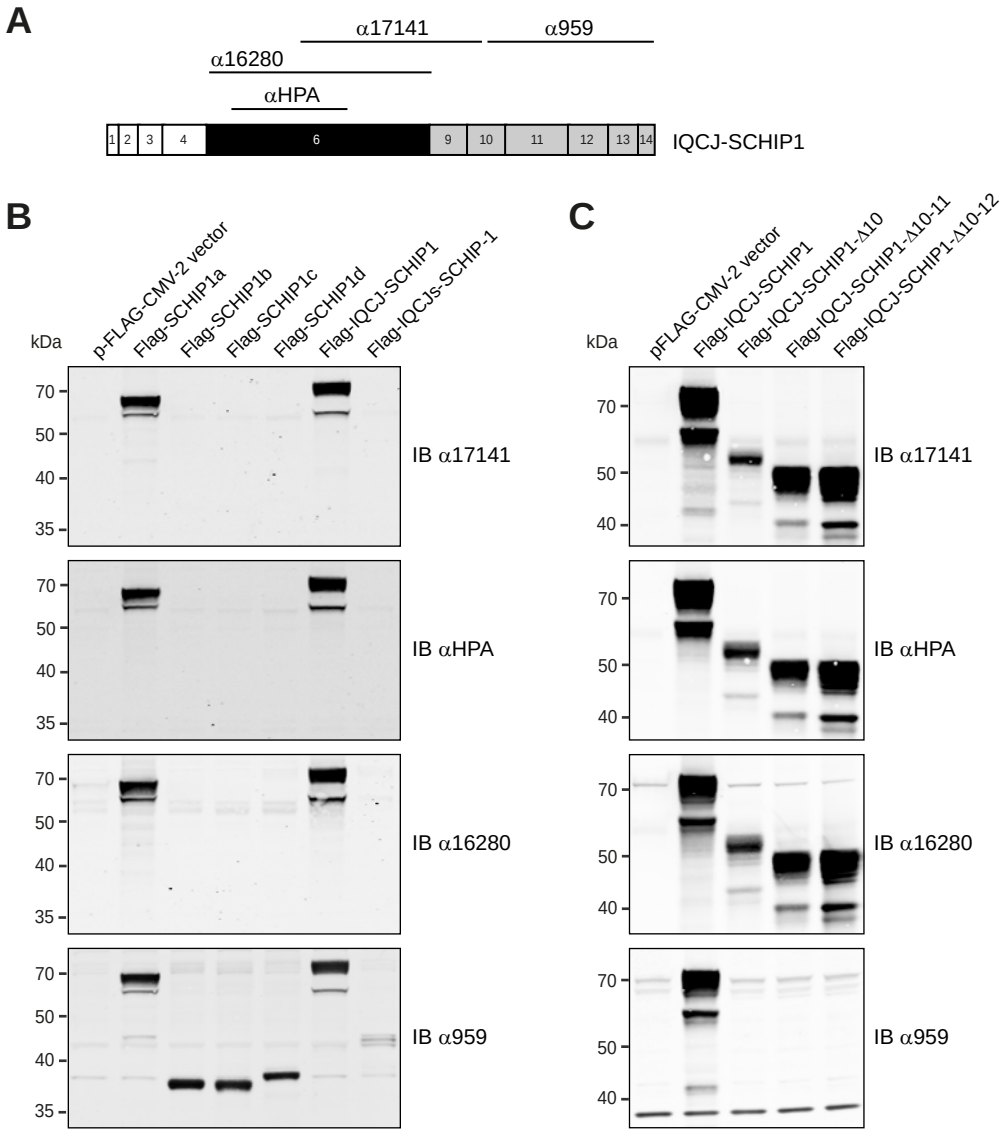

Fig. S2. Characterization of SCHIP1 antibodies. (A) Schematic representation of the antigens used to produce SCHIP1 antibodies (upper lines), aligned along the sequence of the IQCJ-SCHIP1 isoform. Boxes define parts of the protein encoded by the different *Schip1* exons. Grey boxes, exons corresponding to the C-terminal region common to all SCHIP1 isoforms; black box, exon 6 coding for the internal region specific for SCHIP1a and IQCJ-SCHIP1; white boxes, exons 1 to 4 coding for the N-terminal region specific for IQCJ-SCHIP1. (B, C) Characterization of SCHIP1 antibodies by immunoblotting (IB) on lysates from transfected COS-7 cells expressing Flag-tagged SCHIP1 isoforms (Flag-SCHIP1a, Flag-SCHIP1b, Flag-SCHIP1c, Flag-SCHIP1d, Flag-IQCJ-SCHIP1, Flag-IQCJs-SCHIP1) (B), or Flag-tagged IQCJ-SCHIP1 truncated proteins corresponding to the cDNA of IQCJ-SCHIP1 deleted of exon 10 (Flag-IQCJ-SCHIP1-Δ10), exons 10 and 11 (Flag-IQCJ-SCHIP1-Δ10-11), or exons 10 to 12 (Flag-IQCJ-SCHIP1-Δ10-12) (C).

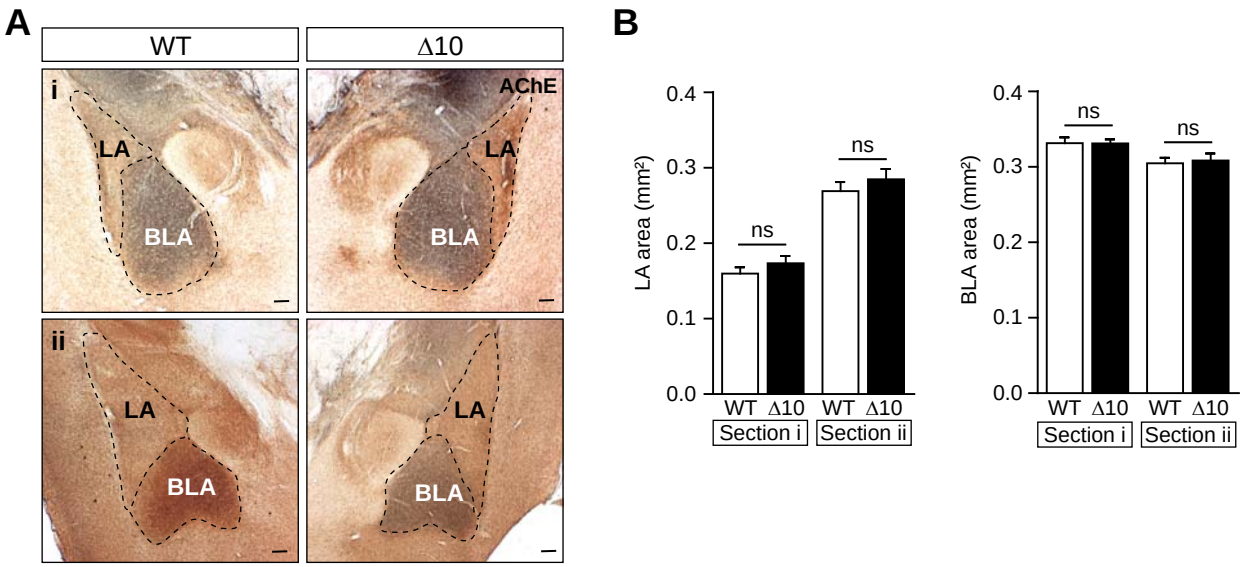

Fig. S3. The size of the amygdala is not affected in *Schip1*Δ10 mice. (A) Acetylcholinesterase (AChE) staining on coronal sections from rostral (i) to caudal (ii) brain regions of adult mice. LA, lateral amygdala; BLA, basolateral amygdala. Black dashed lines show the boundaries of LA and BLA. (B) Quantification of the area of the basolateral and the basal nuclei according to the AChE staining. Data are means±sem. (n=4 animals/genotype; n=3-4 sections/animals; Two-way ANOVA; ns, not significant). Scale bar, 100 µm.

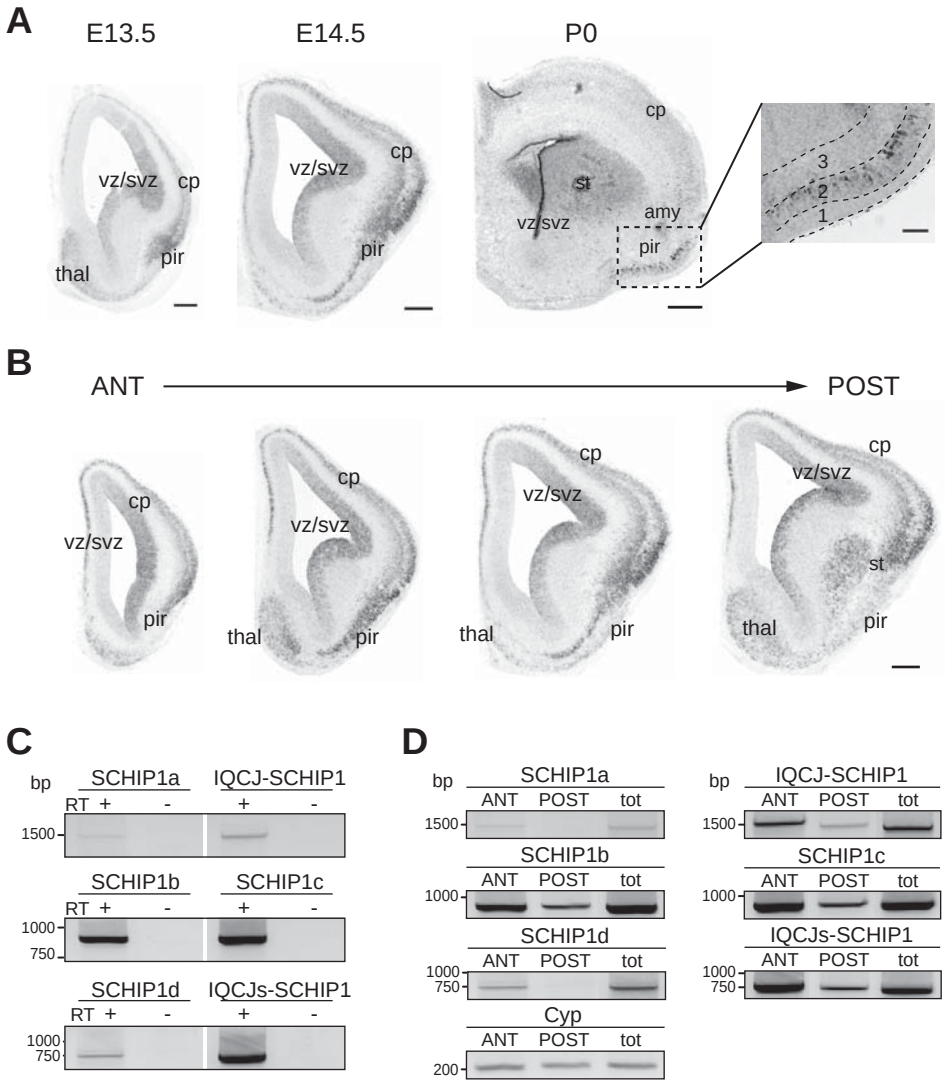

Fig. S4. *Schip1* expression. (A) *In situ* hybridization on WT brain coronal sections at E13.5, E14.5 and P0, using a riboprobe allowing the detection of all *Schip1* mRNA isoforms. Right panel, higher magnification at the level of the piriform cortex at P0. Black dashed lines, boundaries of layers. Pir, piriform cortex; vz/svz, ventricular and subventricular zones; cp, cortical plate; st, striatum; thal, thalamus; amy, amygdala. (B) *In situ* hybridization on WT coronal sections from anterior (ANT) to posterior (POST) regions at E14.5 using a riboprobe allowing the detection of all *Schip1* mRNA isoforms. (C) RT-PCR analysis of the expression of *Schip1* isoforms in WT piriform cortex at E14.5. (D) RT-PCR analysis of the expression of *Schip1* isoforms in WT ANT and POST piriform cortex at E14.5. Cyp, housekeeping gene peptidylpropyl isomerase B. Scale bars, 150 µm.

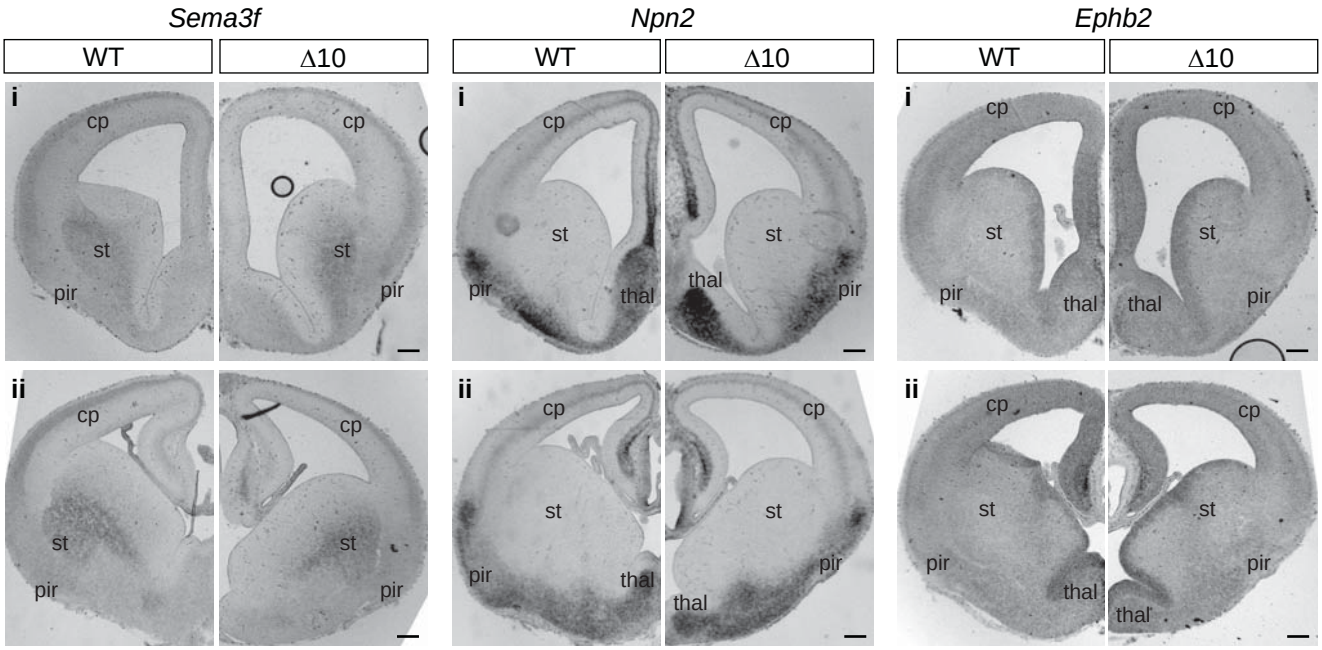

Fig. S5. The expression patterns of *Sema3f*, *Npn2* and *Ephb2* are not affected in *Schip1*Δ10 embryos. *In situ* hybridization on E14.5 brain coronal sections from anterior (i) to posterior (ii) regions using riboprobes allowing the detection of *Sema3f*, *Npn2* and *Ephb2* mRNAs. Pir, piriform cortex; cp, cortical plate; st, striatum; thal, thalamus. Scale bars, 150 µm.

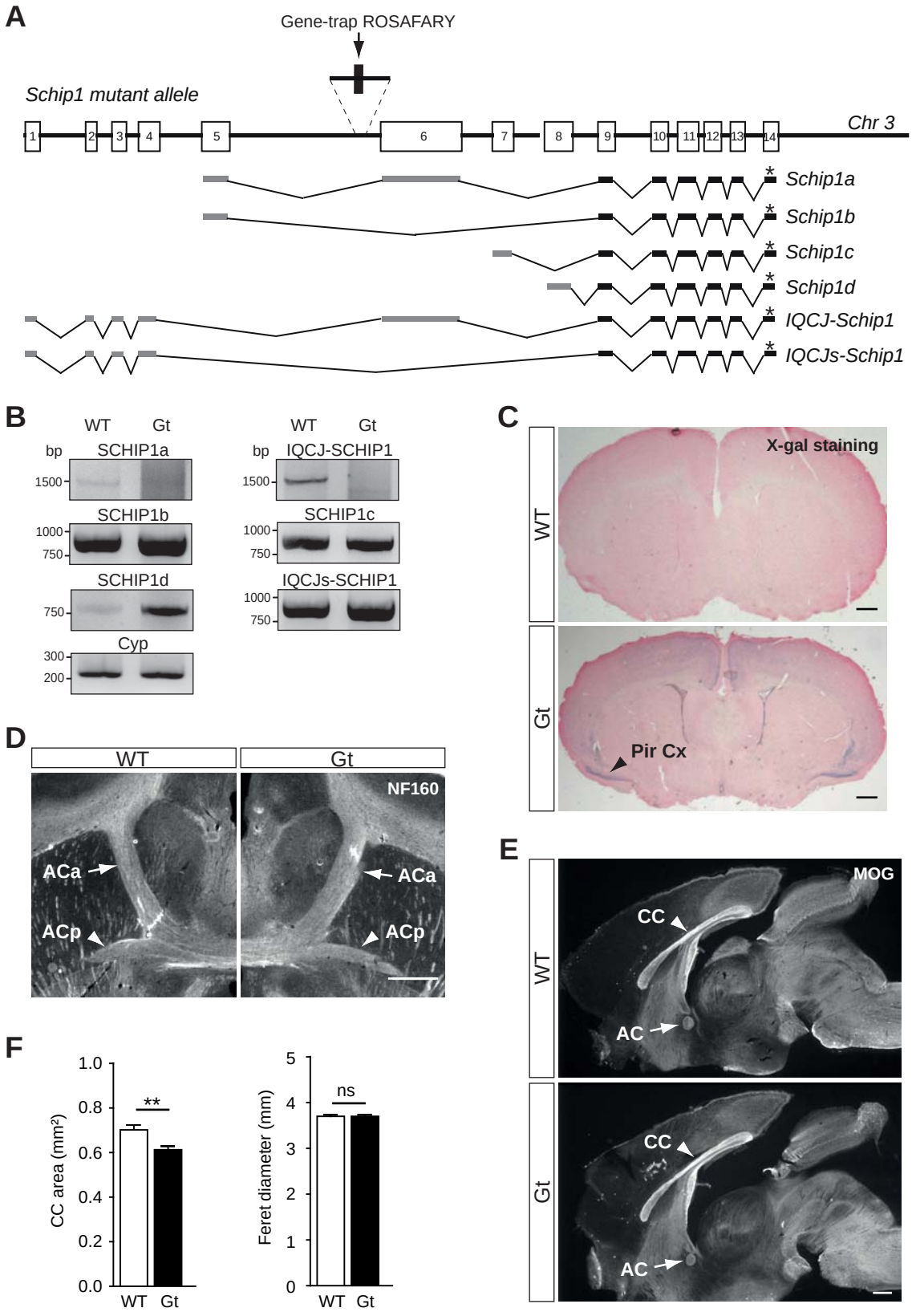

Fig. S6. Characterization of *Schip1*$^{Gt(ROSA)77Sor}$ mice. (A) Schematic representation of *Schip1* gene-trap targeting. The retroviral gene-trap vector ROSAFARY bearing a *lacZ-neo*

reporter fusion gene, was targeted to intron 5 of the *Schip1* gene. (B) Semi-quantitaive RT-PCR analysis of the expression of *Schip1* isoforms in WT and mutant (Gt) adult brains. *Schip1a* and *IQCJ-Schip1* mRNAs are not detectable in Gt mice, whereas the four other isoforms appear overexpressed as compared to WT mice. Cyp, housekeeping gene peptidylpropyl isomerase B. (C) X-gal staining on coronal brain sections detecting the expression of the *lacZ-neo* reporter, notably in the piriform cortex (arrowhead, Pir Cx). (D,E) Gt mice do no present major AC or CC defects. (D) Horizontal brain sections immunolabeled with anti-NF160 antibodies. Arrows, ACa; arrowhead, ACp. (E) Mid-sagittal brain sections immunolabeled with an anti-MOG antibody. Arrows, AC; arrowheads, CC. (F) Quantification of CC area and Feret diameter. Data are means±s.e.m. (n=3 animals/genotype, n=2-3 sections/animal; t-test (G); $^{**}p<0.01$, ns, not significant). Scale bars, 500 µm.

Thickness (µm)	WT	Δ10	P value
P0 ANT neocortex	666.9±26.2	665.8±7.7	*p*=0.9713
P0 POST neocortex	604.2±11.2	586.4±20.6	*p*=0.4669
Adult ANT neocortex	1.131±0.015	1.106±0.017	*p*=0.70
Adult POST neocortex	1.073±0.012	1.031±0.011	*p*=1.00

Table S1: Neocortex thicknesses in newborn and adult mice. Measures were performed on cresyl violet-stained sections at the level of the ACa, named anterior (ANT) region, and at the level of the ACp, named posterior (POST) region. Data are means±s.e.m.; t-test; n=3-5 animals/genotype.

Cell density (x10^3 nuclei/mm^2)	**WT**	**Δ10**	**P value**
ANT piriform cortex layer 2	10.5±0.2	9.9±0.2	*p*=0.14
POST piriform cortex layer 2	9.8±0.2	10.1±0.3	*p*=0.56

Table S2: Piriform cortex layer 2 cell density at P0. Quantification of DAPI stained cells on serial coronal sections. Data are means±s.e.m.; t-test; n=5 animals/genotype.

BrdU+ cell density (cells/mm²)		**WT**	**Δ10**	**P value**
E14.5	ANT piriform cortex	1116±94	1139±80	*p*=0.59
	POST piriform cortex	721±71	769±43	*p*=0.33
E18.5	ANT piriform cortex layer 2	1330±70	1343±56	*p*=0.87
	POST piriform cortex layer 2	1120±53	1143±92	*p*=0.91
Layer 2 thickness (µm)		**WT**	**Δ10**	**P value**
E18.5	ANT piriform cortex layer 2	79.8±4.5	68.7±2.6	*p*=0.098
	POST piriform cortex layer 2	73.0±2.4	63.7±2.2	***p*=0.046***

Table S3: BrdU immunopositive cell density in the piriform cortex at E14.5 and E18.5 and layer 2 thickness at E18.5. Quantification of BrdU immunopositive cell density in the whole piriform cortex at E14.5 and in the piriform cortex layer 2 at E18.5, after BrdU injection in pregnant females at E12.5. Data are means±s.e.m.; t-test; n=3 animals/genotype.

Neuron density (Tuj-1+ cells/field)	**WT**	**Δ10**	**P value**
DIV2	34.1±1.6	33.3±1.1	*p*=0.69
DIV4	40.8±1.5	43.9±2.2	*p*=0.25
Cleaved caspase 3+ cells (%)	**WT**	**Δ10**	**P value**
DIV1	2.8±0.27	2.3±0.39	*p*=0.33
DIV2	4.6±0.69	4.4±0.67	*p*=0.84
DIV4	2.8±0.55	3.0±0.35	*p*=0.70
DIV6	3.8±1.11	2.1±0.33	*p*=0.15

Table S4: Neuron density and percentage of cleaved caspase 3 immunopositive cells in cultures of dissociated piriform cortex neurons. Quantification of Tuj-1 stained cells per acquisition (20X magnification); n=10 acquisitions/embryo, n=5 embryos/genotype (DIV2), n=3 embryos/genotype (DIV4). Percentage of cleaved caspase 3 immunopositive cells per acquisition (20X magnification); n=10 acquisitions/embryo, n=3 embryos/genotype (DIV1), n=5 embryos/genotype (DIV2), n=5-9 embryos/genotype (DIV4), n=3 embryos/genotype (DIV6) . Data are means±s.e.m.; t-test.

Antigen	Species	Reference	Use	Dilution
β3 tubulin	mouse	Covance, #MMS-435P (TUJ1)	IF	1:1000
NF160	mouse	Sigma, #N5264	ICH	1:250
L1CAM	rat	Millipore, #5272	ICH	1:200
MOG	Mouse	Millipore, #5680	ICH	1:50
cleaved caspase 3	rabbit	BD Biosciences, #559565	IF/ICH	1:250
human IgG1	goat	Vector Laboratories, #J0307	IF	1:250
BrdU	rat	ABcys, #117-7513	ICH	1:1000
Flag	rabbit	Sigma, #F7425	IF	1:250
Neuropilin 2	goat	R&D system, #AF567	IF	1:300
digoxigenin (DIG) (alkaline phosphatase-coupled)	sheep	Roche, #11093 274910	ICH	1:2500
Cy3 and Alexa Fluor 488/546/633 conjugated-secondary antibodies	Goat Donkey	Molecular Probes	IF/ICH	1:800
IRDye^{TM}800CW and IRDye^{TM}680CW secondary antibodies	Goat	Rockland Immunochemicals	IB	1:10000

Table S5: Primary and secondary antibody characteristics. IF, immunolabeling on cultured neurons; ICH, immunostaining on tissue; IP, immunoprecipitation; IB, immunoblot.