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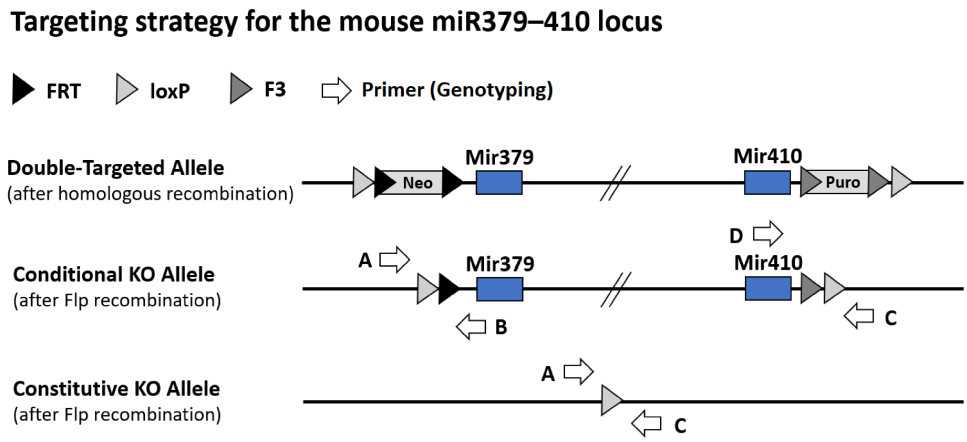
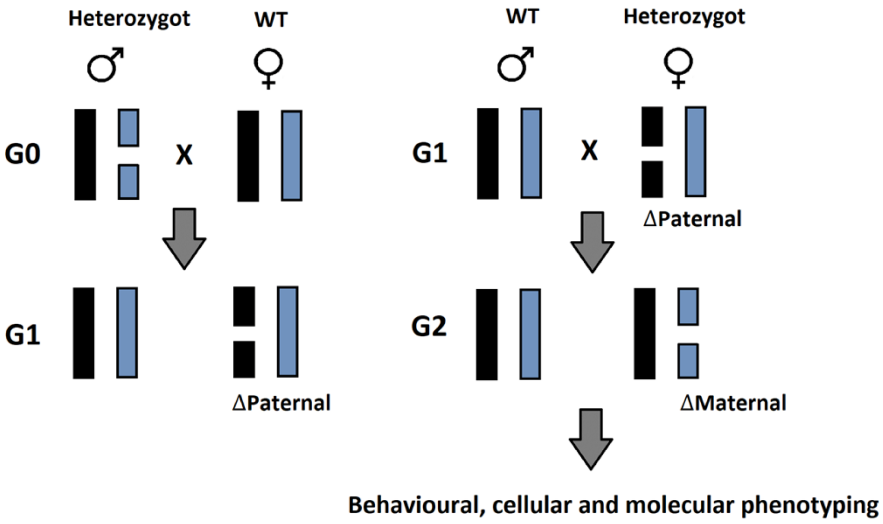
Appendix Figures

Figure S1

a



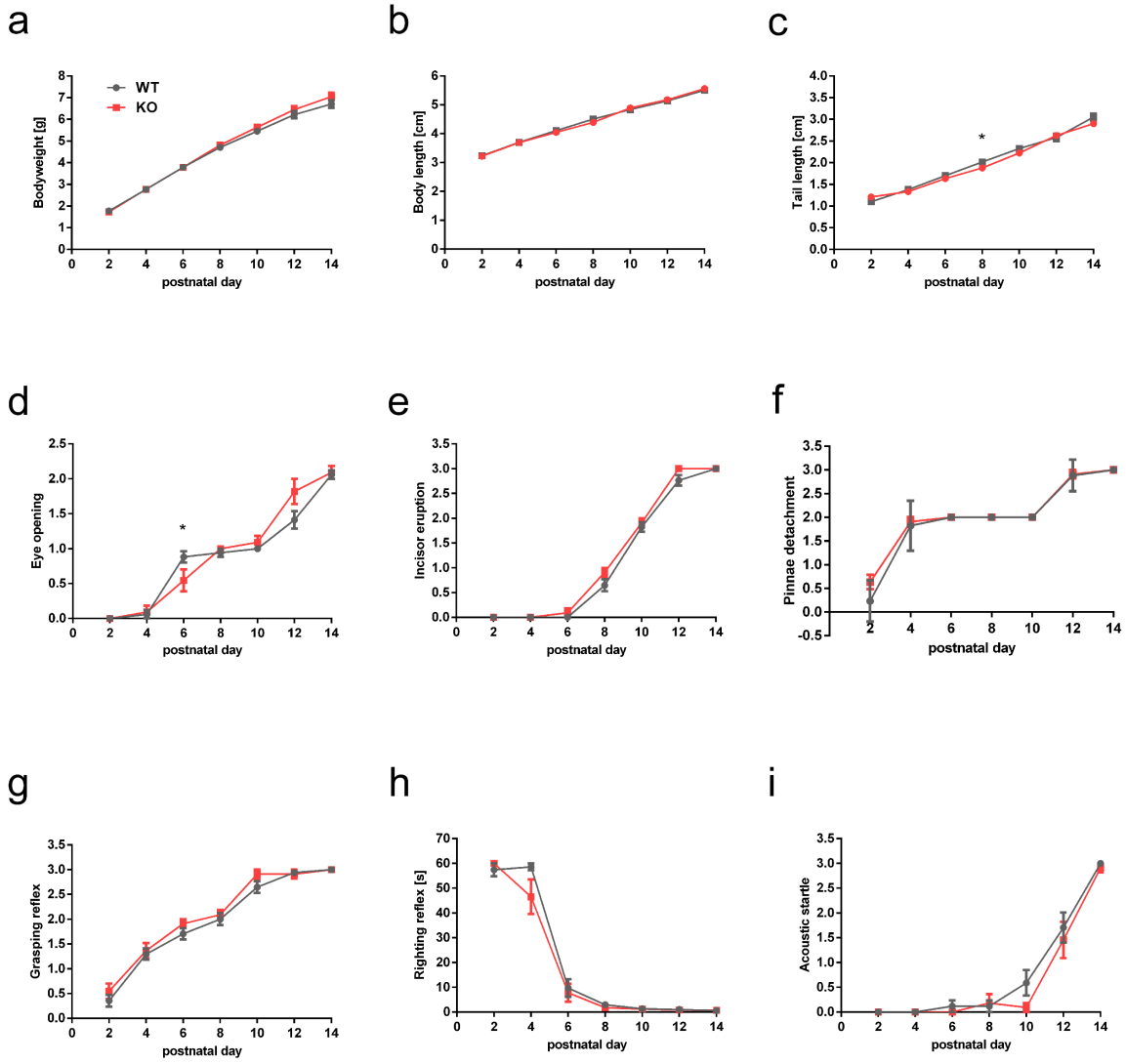
b



Appendix Fig. S1: The miR379-410 mouse model. a, Schematic illustration of the targeting strategy for the mouse miR379-410 locus as described previously in more details (Valluy et al. 2015). Target sequences of recombinases and the location of primers (A-D) used for genotyping are indicated. **b**, A schematic representation of the breeding strategy for behavioural, cellular and molecular phenotyping of miR379-410 wt / ko mice (G2) is shown. Please note that the miR379-410 cluster is paternally imprinted and therefore only expressed from the maternal allele (Seitz et al. 2004). To study the physiological relevance of the cluster, wildtype (wt) male were bred with

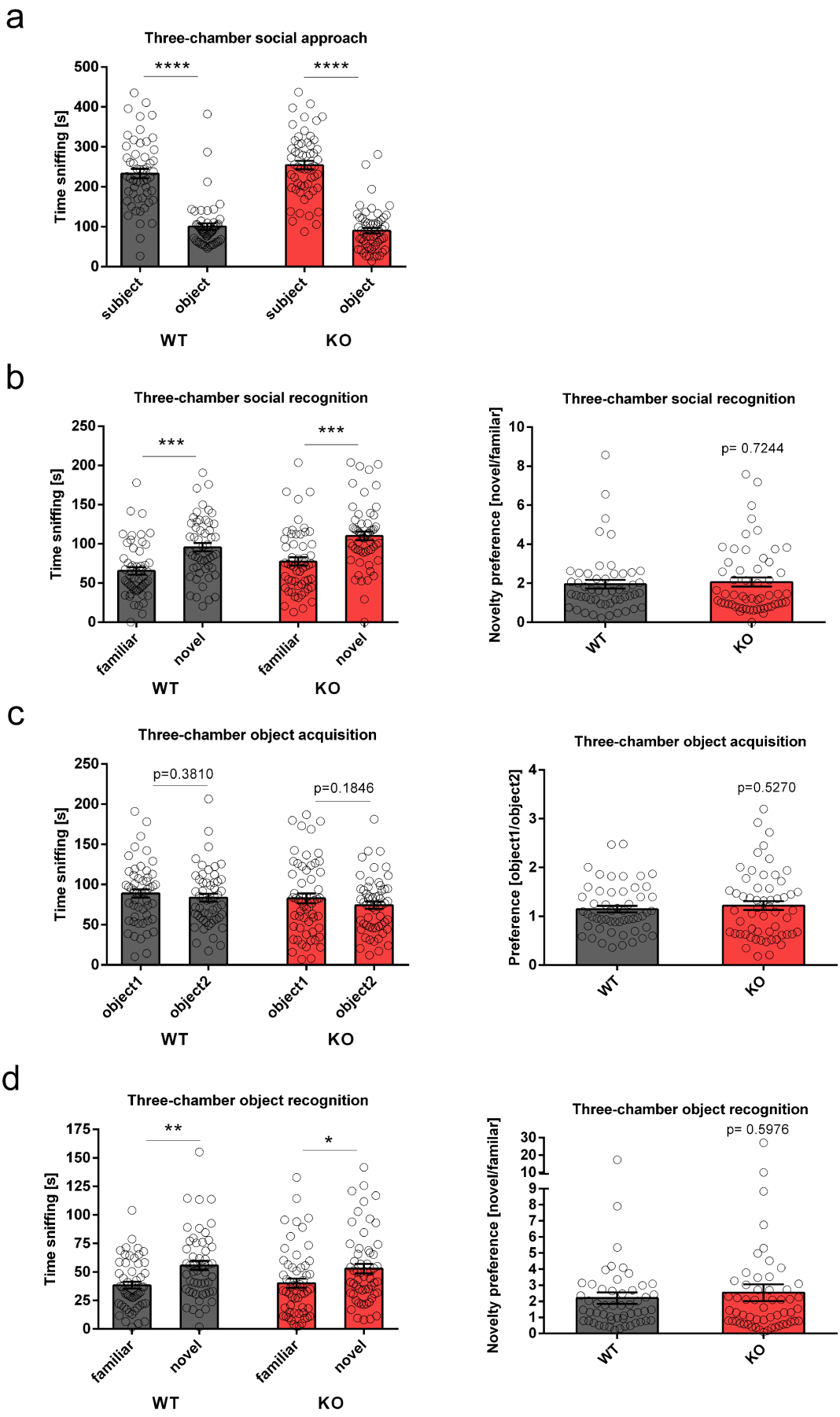
miR379-410 heterozygous mutant female mice (G1), that carried the mutation on the paternal allele (ΔPaternal) to obtain mice deficient for the miR379-410 cluster. Heterozygous mice resulting from this breeding (G2) carried the deletion on the maternal allele (ΔMaternal). Therefore, the miR379-410 cluster is not expressed in these animals (in the following named as “ko” mice) as previously demonstrated (Valluy et al. 2015; Marty et al. 2016). Abbreviation: G=Generation.

Figure S2



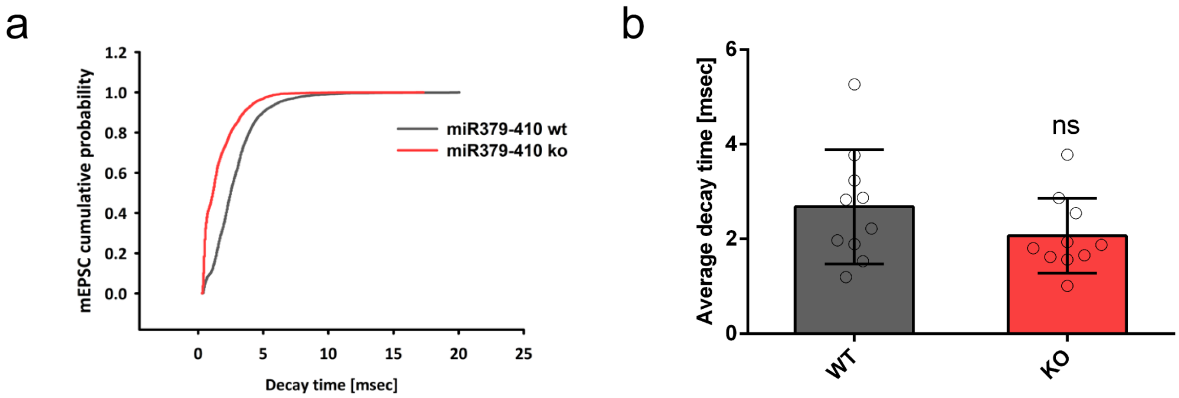
Appendix Fig. S2: Developmental milestones: Somatic growth and neurological reflexes in miR379-410 wt / ko mouse pups. miR379-410 ko pups displayed no delays on several milestones during early development (P 2-14). **a**, Body weight. **b**, body length. **c**, tail length, development x genotype: $F_{6,156}$=2.699, p=0.016, rm-ANOVA; P 8 t_{26}=2.581, *p=0.0158, unpaired Student`s t-test. **d**, eye opening, development x genotype: $F_{6,156}$=3.279, p=0.005, rm-ANOVA; P 6 $t_{26}$=2.095, *p=0.0461, unpaired Student`s t-test. **e**, incisor eruption. **f**, pinnae detachment. **g**, grasping reflex. **h**, righting reflex, development x genotype: $F_{6,156}$=2.119, p=0.054, rm-ANOVA. **i**, acoustic startle. Grey line: miR379-410 wildtype littermate control n=17 (male n=8, female n=9); red line: miR379-410 ko mice n=11 (male n=5, female n=6). Data are presented as mean ± s.e.m.

Figure S3


a
Three-chamber social approach
Time sniffing [s]
****
****
subject
object
subject
object
WT
KO
b
Three-chamber social recognition
Time sniffing [s]
***
***
familiar
novel
familiar
novel
WT
KO
Three-chamber social recognition
Novelty preference [novel/familar]
p= 0.7244
WT
KO
c
Three-chamber object acquisition
Time sniffing [s]
p=0.3810
p=0.1846
object1
object2
object1
object2
WT
KO
Three-chamber object acquisition
Preference [object1/object2]
p=0.5270
WT
KO
d
Three-chamber object recognition
Time sniffing [s]
**
*
familiar
novel
familiar
novel
WT
KO
Three-chamber object recognition
Novelty preference [novel/familar]
p= 0.5976
WT
KO

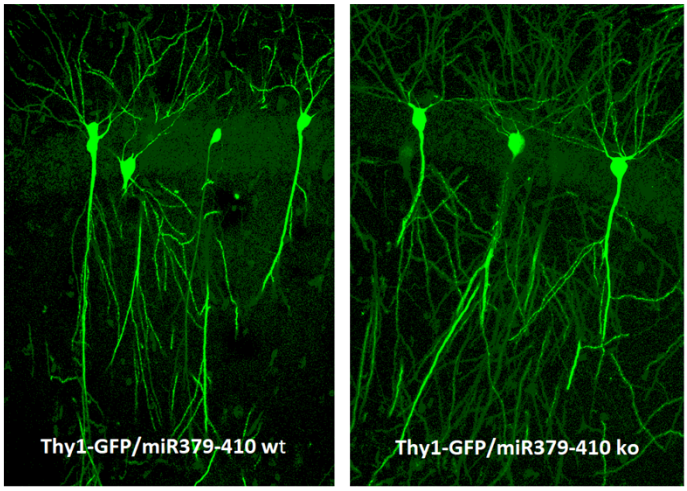
Appendix Fig. S3: Social and object memory was not affected in miR379-410 ko mice. a-b, Three-chamber social memory test in adolescent mice. **a**, Three-chamber social approach test for adolescent miR379-410 wt and ko mice. Time sniffing subject and object is shown, wt n=53 (male n=26, female n=27), t_{52}=7.444, ****p<0.0001; ko n=56 (male n=26, female n=30), t_{55}=10.16, ****p<0.0001; paired Student`s t-test. **b**, Three-chamber social recognition test. **b, left panel:** Time sniffing familiar and novel animal is shown for adolescent miR379-410 wt mice n=53 (male n=26, female n=27), t_{52}=3.976, ***p<0.0002; and ko mice n=56 (male n=26, female n=30), t_{55}=3.557, ***p<0.0008; paired Student`s t-test. **b, right panel:** Novelty social preference index in adolescent mice - defined as the ratio of time sniffing a stranger mouse vs. a familiar mouse - is shown, wt n=52 (male n=25, female n=27), ko n=56 (male n=26, female n=30), $t_{106}$=0.3535, ns p=0.7244; unpaired Student`s t-test. **c-d**, Three-chamber object memory test in adolescent mice. **c, left panel:** Object acquisition test. Time sniffing identical object1 and object2 are shown, wt n=53 (male n=26, female n=27), t_{52}=0.8836, p=0.3810; ko n=57 (male n=26, female n=31), t_{56}=1.343, p=0.1846; paired Student`s t-test. **c, right panel:** Preference index in adolescent mice - defined as the ratio of time sniffing object1 vs. object2 - is shown, wt n=53 (male n=26, female n=27), ko n=57 (male n=26, female n=31), $t_{108}$=0.6347, ns p=0.5270; unpaired Student`s t-test. **d, left panel:** Object recognition task. Time sniffing familiar and novel object are shown, wt n=53 (male n=26, female n=27), t_{52}=3.282, **p=0.0018; ko n=57 (male n=26, female n=31), t_{56}=2.042, *p=0.0459; paired Student`s t-test. **d, right panel:** Novelty object preference index in adolescent mice - defined as the ratio of time sniffing a novel object vs. a familiar object - is shown, wt n=53 (male n=26, female n=27), ko n=56 (male n=25, female n=31), $t_{107}$=0.5295, ns p=0.5976; unpaired Student`s t-test. Data are presented as mean ± s.e.m.

Figure S4



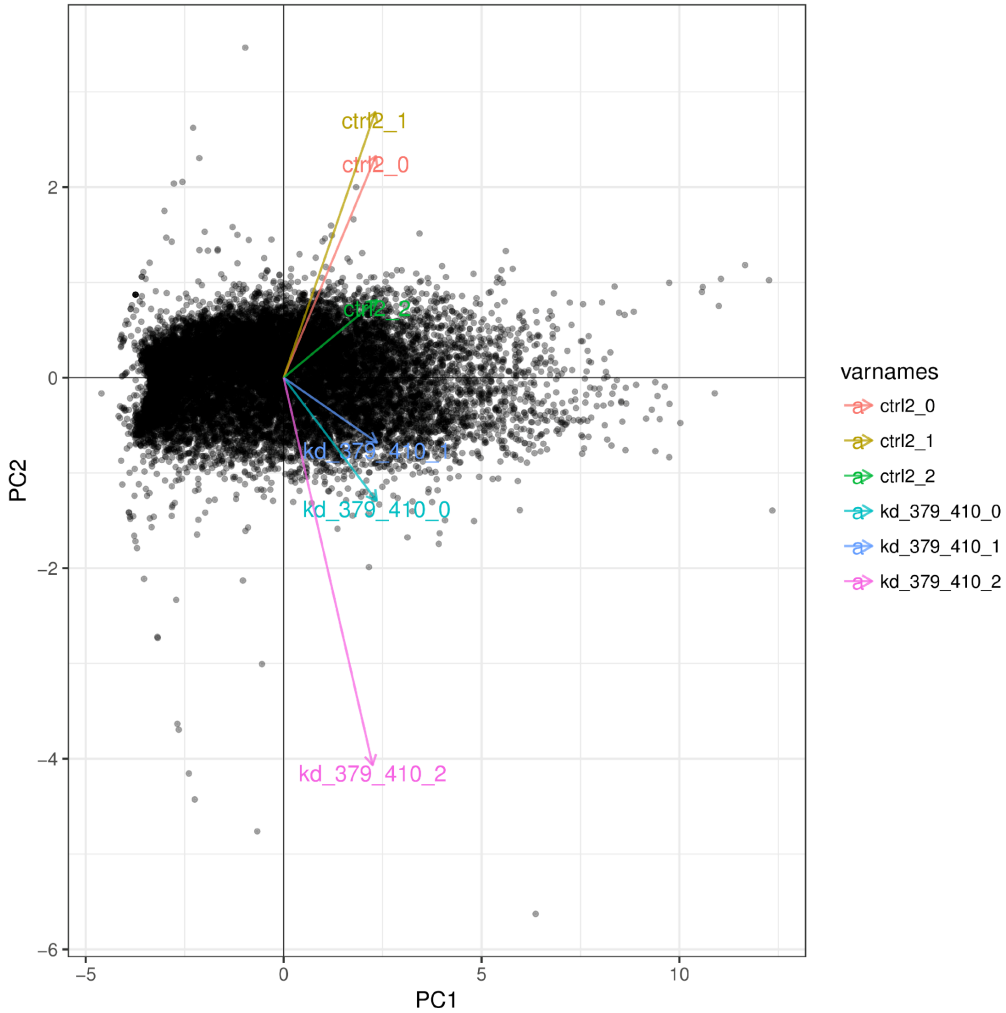
Appendix Fig. S4: mEPSC decay time was unchanged in miR379-410 ko neuronal culture. a, Cumulative decay time measured in primary hippocampal neurons (DIV8-10) isolated from miR379-410 wt and ko mice, wt n=10, ko n=10 cells analyzed. **b**, Average decay time measured in primary hippocampal neurons (DIV8-10) isolated from miR379-410 wt and ko mice, wt n=10, ko n=10, t_{18}=1.342, ns p=0.1963; unpaired Student`s t-test. Data are presented as mean ± s.d.

Figure S5



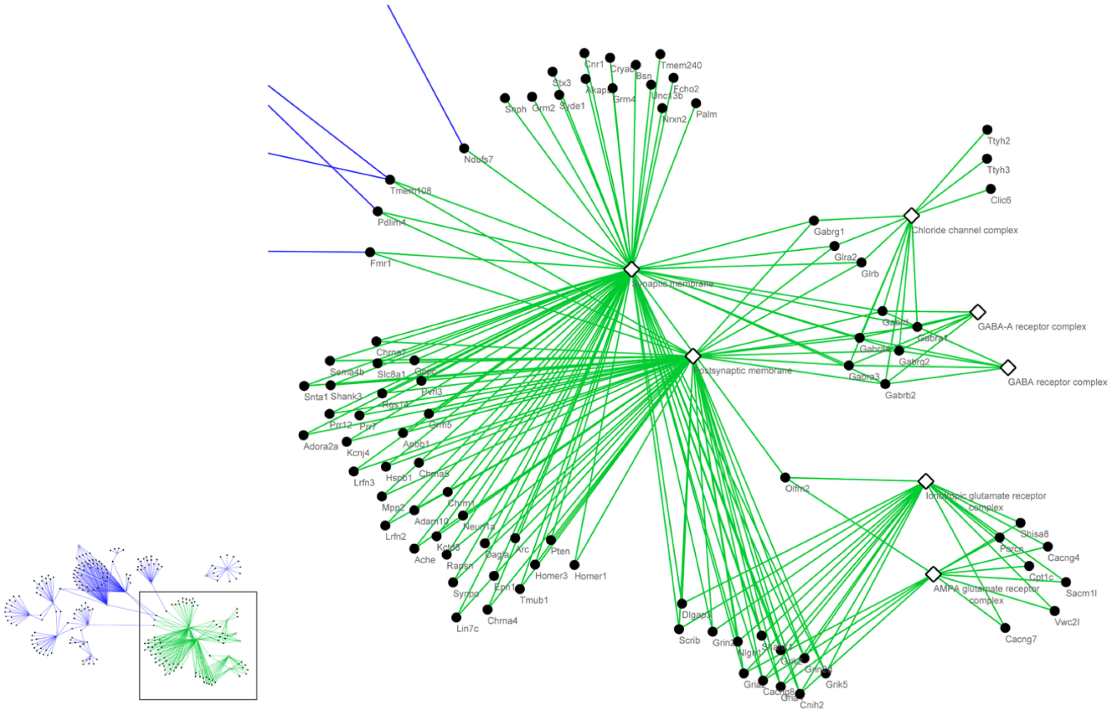
Appendix Fig. S5: Hippocampal expression of Thy1-GFP/miR379-410 wt and ko mice. Neuronal health and dendritic complexity were visibly not impaired in adult miR379-410 ko mice. Confocal images of Thy1-GFP immunofluorescence were examined over 50 µm-thick coronal sections. Hippocampal CA1 regions of adult male miR379-410 wt or ko mice are shown.

Figure S6



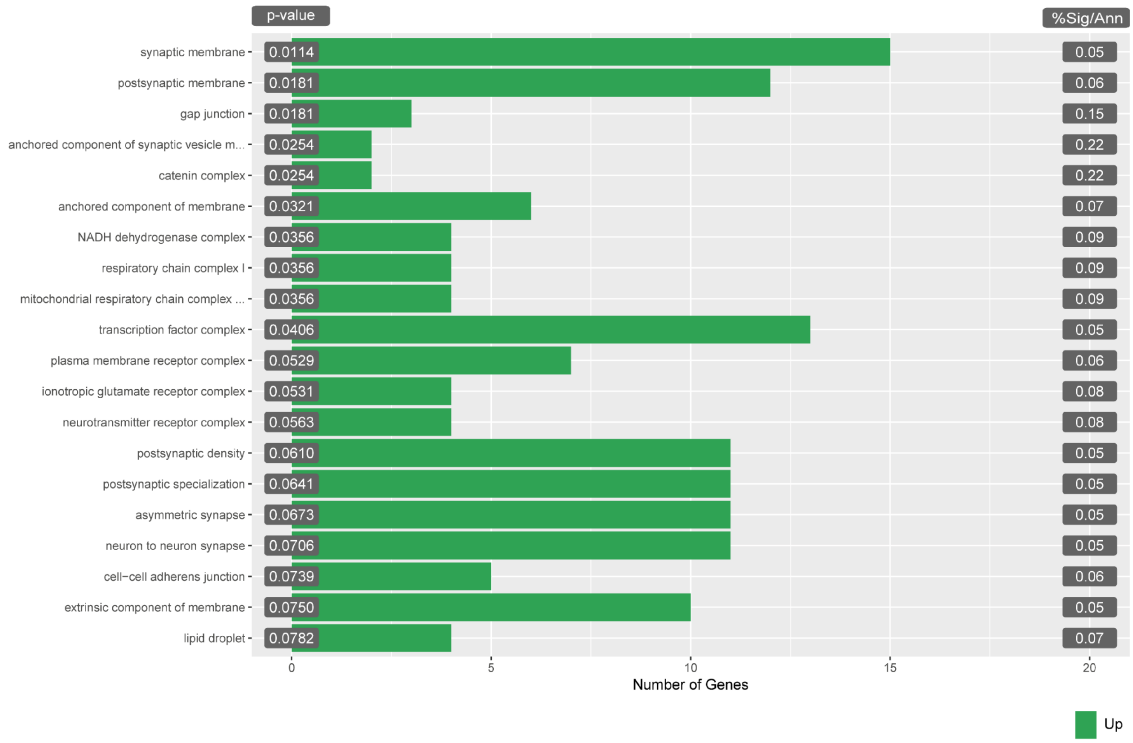
Appendix Fig. S6: Principal component analysis (PCA) for RNAseq gene expression data. PCA of gene-expression data from all miR379-410 wt (ctrl2_0, ctrl2_1, ctrl2_2) and ko (kd_379_410_0, kd_379_410_1, kd_379_410_2) shows a segregation of biological replicates into controls and miR379-410 ko conditions. Abbreviations: PC1, x-axis (Principal Component 1); PC2, y-axis (Principal Component 2).

Figure S7



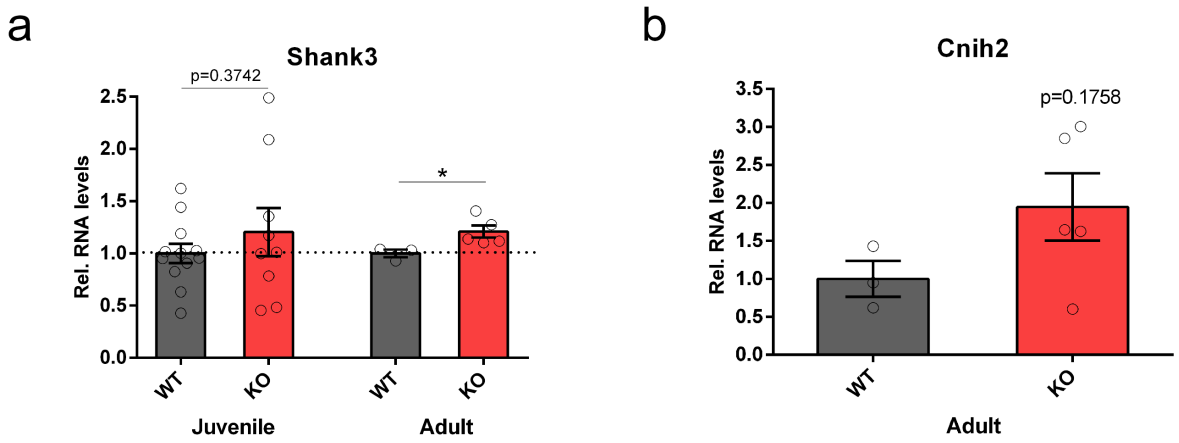
Appendix Fig. S7: Bipartite network analysis. Network representation of detected GO-Terms (cellular component) and associated genes reveals a synaptic cluster highlighted in green. GO-Terms with more than 300 annotated genes are filtered out for reasons of clarity and comprehensibility

Figure S8



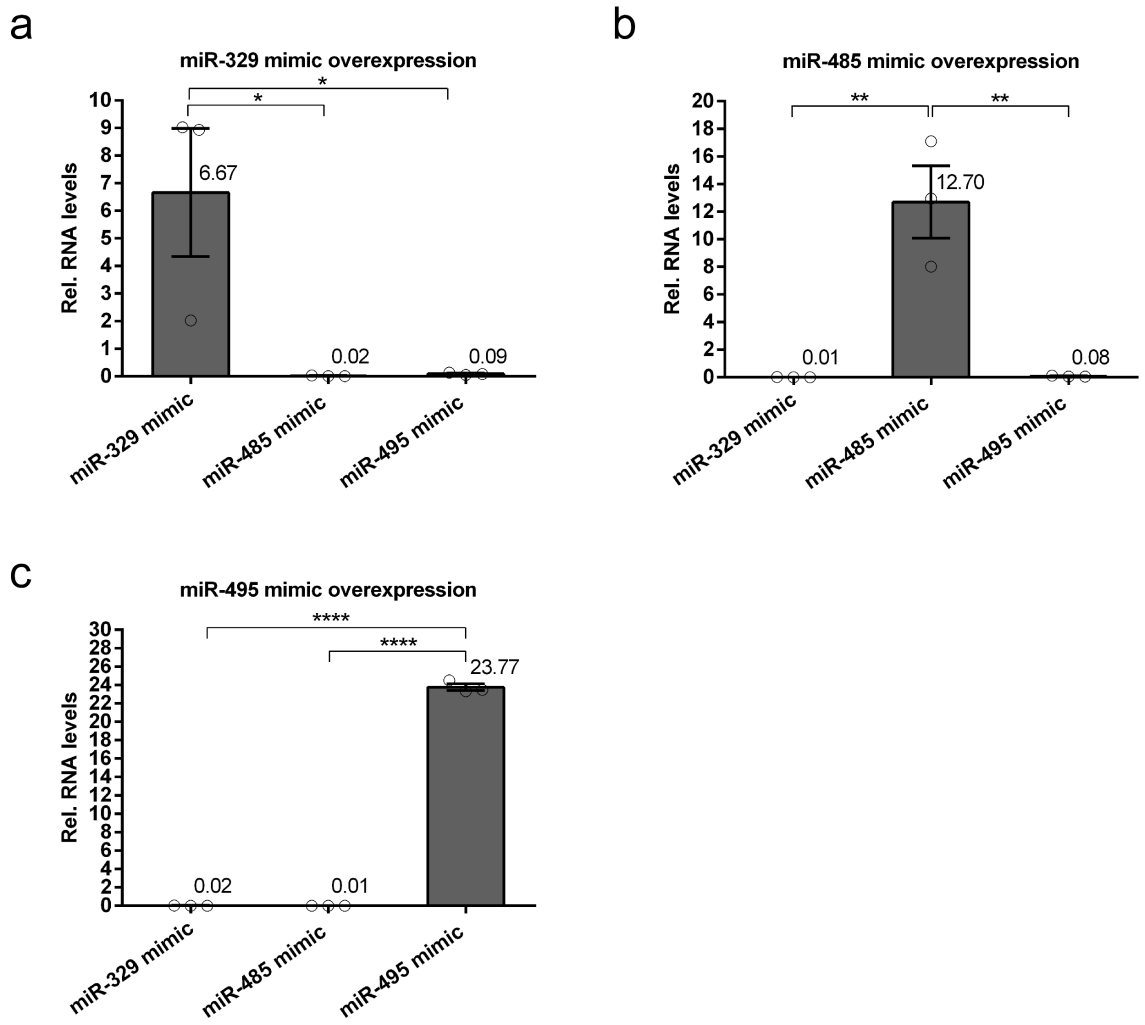
Appendix Fig. S8: GO-Term enrichment analysis of upregulated DEGs with miR379-410 binding site motifs. Green bars indicate the percentage of upregulated genes containing a 379-410 cluster miRNA binding site associated with specific GO-Terms; significant upregulation at p-value $p<0.05$.

Figure S9



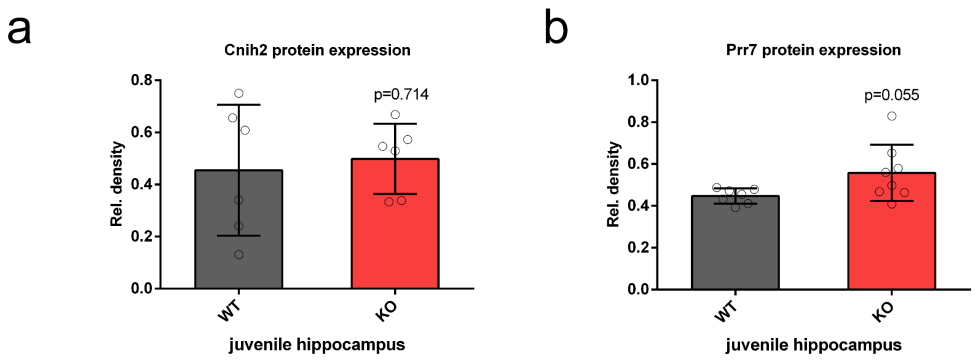
Appendix Fig. S9: qPCR expression levels of hippocampal RNA samples in miR379-410 wt / ko mice. a, qPCR validation of predicted direct miR379-410 target Shank3 in male juvenile (wt n=7, ko n=4, t_9= 0.4671, ns p=0.6515) and adult (wt n=3, ko n=5, t_6=2.538, *p=0.0442, as shown in Fig. 4b) hippocampi of miR379-410 wt / ko mice; unpaired Student`s t-test. **b,** qPCR validation of predicted direct miR379-410 target Cnih2 in male adult (wt n=3, ko n=5, $t_6$=1.534, *p=0.1758) hippocampi of miR379-410 wt / ko mice; unpaired Student`s t-test. Data are presented as mean ± s.e.m.

Figure S10



Appendix Fig. S10: qPCR expression of mature miRNAs in mimic transfected rat cortical neurons. Rat cortical neurons were transfected with the indicated mimics (DIV12-15) and expression level of the mature miRNA were quantified by qPCR **a,** miR-329 mimic overexpression (miR-329 mimic vs. miR-485 mimic, t_4=2.867, *p=0.0456; miR-329 mimic vs. miR-485 mimic, t_4=0.0471, *p=0.0471; n=3 per condition, unpaired Student`s t-test). **b,** miR-485 mimic overexpression (miR-329 mimic vs. miR-485 mimic, $t_4$=4.834, **p=0.0084; miR-485 mimic vs. miR-495 mimic, $t_4$=4.807, **p=0.0086; n=3 per condition, unpaired Student`s t-test). **c,** miR-495 mimic overexpression (miR-329 mimic vs. miR-495 mimic, t_4=64.85, ****p<0.0001; miR-485 mimic vs. miR-495 mimic, t_4=64.87, ****p<0.0001; n=3 per condition, unpaired Student`s t-test). Data presented as mean ± s.e.m.

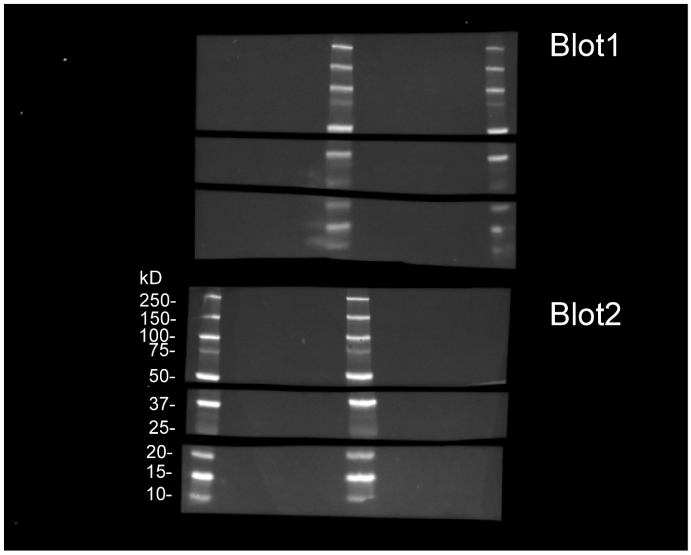
Figure S11



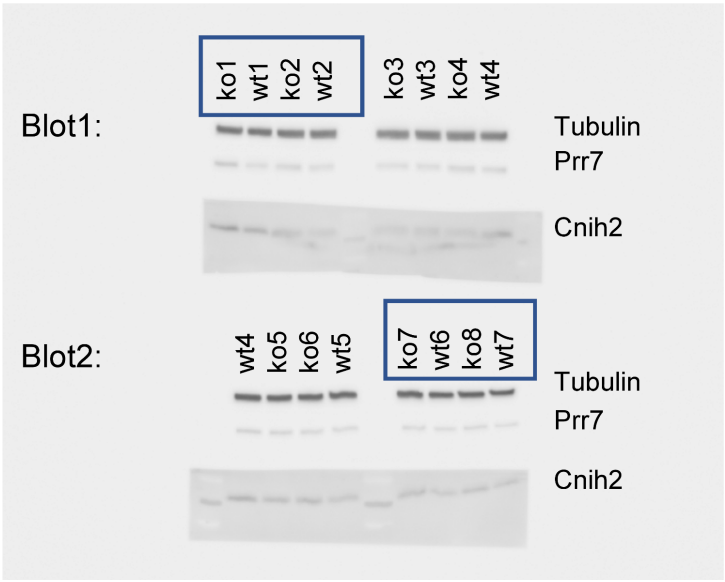
Appendix Fig. S11: Protein expression level of Cnih2 and Prr7 normalized to Tubulin in juvenile miR379-410 wt / ko hippocampus. a-b, Western blot quantification of Cnih2 and Prr7 normalized to loading control Tubulin. **a,** Cnih2 protein expression, wt n=6, ko n=6, t_{10}=0.3768, p=0.7124, unpaired Student`s t-test. **b,** Prr7 protein expression, wt n=7, ko n=8, $t_{13}$=2.105, p=0.0553, unpaired Student`s t-test. Data are presented as mean ± s.d.

Figure S12

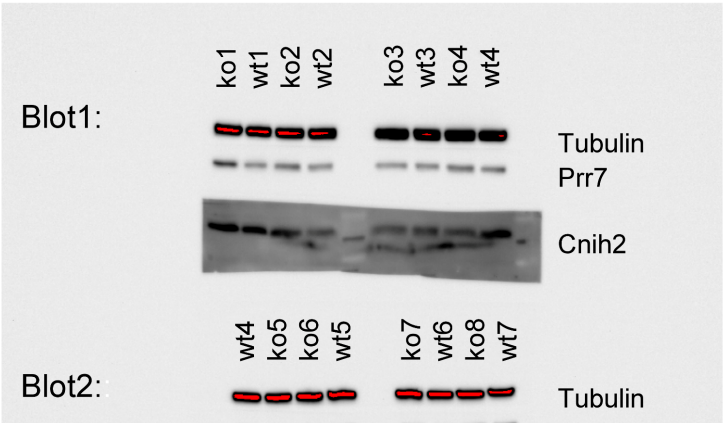
a



b



b‘ (saturated)



Appendix Fig. S12: Full-length blots for Cnih2, Prr7 and Tubulin related to Fig. EV5. a, Membranes were cut after blotting to simultaneously probe for proteins Tubulin, Prr7 and Cnih2 running at different molecular weights. **b-b',** Full-length blots for Tubulin, Prr7 and Cnih2. Blue boxes indicate regions of blot presented in Fig. EV5 for Cnih2 (Fig. EV5 b) and Prr7 (Fig. EV5 c).

Supplementary methods

Developmental milestones and somatosensory reflexes

The tests were examined between 09:00–14:00h during the light phase of the 12 h/12 h light/dark cycle. Each pup was tested at approximately the same time of day. Every other day from P 2–14, body weight, body length and taillength was recorded. Body weight was measured using a palmscale (PS6-250; My Weigh Europe, Hückelhoven, Germany). The following physical landmarks were also recorded: Pinnae detachment, eye opening, incisor eruption. Somatosensory reflexes, surface righting, grasping reflexes and auditory startle (acoustic stimulus: hand clapping) were scored. Latencies were measured in seconds for surface righting (maximum: 60 s). Other somatic and behavioural variables were rated semi-quantitatively, as followed: 0 = no response/not present, 1 =slight response/slightly present, 2 = strong response/strongly present, 3 = complete response/completely present.

Ultrasonic vocalizations in isolated pups

Subject pups were isolated from their mother and littermates for 10 min under room temperature (20–23°C). Pups were randomly selected and individually removed from the nest and gently placed into a glass isolation container, filled with fresh bedding material. The isolation container was surrounded by a sound attenuating Styrofoam box. USV emission was monitored by an Ultra Sound Gate Condenser Microphone CM16, sensitive to frequencies of 15–180 kHz (flat frequency response between 25 and 140 kHz; ±6 dB; Avisoft Bioacoustics, Berlin, Germany), that was placed in the roof of the sound attenuating box, 22 cm above the floor. Emitted USV calls from the pups were recorded and analyzed afterwards by a trained person.

Elevated pus maze

Adult mice were tested in an elevated plus-maze, consisted of two open arms and two closed arms extending from a central area. Room light was approximately set to 30 lux. Mice were individually placed in the center, facing one of the closed arms. The subject was allowed to freely explore the maze for 5 min. Time spent in the open arms and numbers of entries into the open arms were recorded automatically by the TSE VideoMot2 analyzer software (TSE Systems, Bad Homburg, Germany).

Marble burying test

A standard plastic cage was equidistantly arranged with 20 glass marbles (diameter size: 15 mm) in a 4 × 5 arrangement on top of a 4.5 cm layer of fresh bedding material. Mice were tested for a duration of 30 min. and the numbers of marbles buried (half and completely) were recorded. Two animals were identified as outliers (interquartile range: 1.5xIQR) by SPSS and excluded from the analysis.

Reciprocal social interaction and USV analysis

In addition, USV emission of juvenile pairs during the social interaction was examined by an Ultra Sound Gate Condenser Microphone CM16 that were placed 20 cm above the testing cage. Before testing the social interaction of mice-pairs, one of the two subjects were placed alone for one minute in the neutral cage, for USV baseline recordings, before the other was placed to it. USV were analyzed with Avisoft-SAS Lab Pro software (Version 5.2.05; Avisoft Bioacoustics). A fast Fourier transform was conducted (512 FFT length, frame size: 100%, Hamming Window and 75%-time window overlap), producing spectrograms at 488 Hz frequency resolution and 0.512 ms temporal resolution. USV were marked and counted by a trained observer blind to genotypes.

Three-chamber box

In brief, behavioural testing was conducted on three consecutive days. On day 1 (habituation phase), subject mice were individually kept for 30 min in a Makrolon type III IVC cage (isolation period) and was then allowed to explore the empty three chambered box for 30 min for habituation. On day 2 and day 3, subject mice were again kept first individually for 30 min (isolation period) and after it, the social behaviour assay (social memory) and the non-social memory assay (object memory) were performed in a balanced order.

Three-chamber social memory

After isolation period for 30 min, subjects were tested for sociability (social approach) and social memory (social recognition). The assay consists of three phases: the social approach trial (10 min), the inter-trial interval (30 min break), and the social recognition

trial (10 min). In the social approach trial, each individual mouse was allowed to freely explore for 10 min the three-chambered box, containing an empty wired-cage (object) on one side and a stimulus mouse constrained in an identical wired-cage (stranger) on the other side. After the social approach trial, the test subject was individually kept for 30 min in the previously used Makrolon type III IVC cage for the inter-trial interval break. After a 30 min delay, the subject was returned to the three-chambered box for a 10 min social recognition trial. During the social recognition trial, subject mice were given the choice between the stimulus mouse (familiar) from the previous social approach trial on the same side where it has been presented before and a novel stimulus mouse (novel) replacing the empty wired-cage. Stimulus mice were age- and sex-matched C57BL/6N mice (Charles River Laboratories, Sulzfeld, Germany) and were group-housed under similar conditions as subject mice and habituated to the wired-cages for 30 min prior testing as well. Location and stimulus mice used in this assay were counter-balanced between subject testing mice.

Three-chamber object memory

After an isolation period of 30 min, subjects were tested for object memory (object recognition). The assay consists of three trial phases: the object acquisition trial (10 min), the inter-trial interval (30 min break), and the object memory trial (10 min). In the object acquisition trial, each subject mouse was allowed to freely explore for 10 min the three-chambered box containing two identical sample objects, which were centrally placed in each of the two side chambers. After the object acquisition trial, the subject was individually kept for 30 min in the previously used Makrolon type III IVC cage for the inter-trial interval break before being placed again in the middle of the three-chamber box. For the object memory trial, one of the old (familiar) objects was replaced with a novel object of similar size but different in colour, shape and material (novel object) to test object recognition memory. In the object memory trial, each subject was allowed to freely explore for 10 min the three-chambered box containing two different sample objects, which were centrally placed in each of the two side chambers. Location and type of objects presented were counter-balanced between subject mice.

Behaviour analysis

For the three-chamber social and object memory test, time spent within each chamber and time spent with subject/object investigation were examined from videos using

Noldus Observer XT software (Noldus Information Technology, Wageningen, The Netherlands) on a personal computer by a trained observer blind to genotypes. Object investigation was defined as time spent sniffing the social stimulus/object when the nose was oriented towards it, i.e. with the nose object distance being 3 cm or less. Social and object recognition (novelty) were defined as spending significantly more time sniffing the novel than the familiar object or mouse, respectively. For the three-chamber social and object memory assay, behaviour recorded within the first 5 min of each trial was included in the statistical analysis, as described previously[1]. Please notice the following, concerning animals' numbers: One ko female was not able to perform the social memory assay since it was outside the test field, one wt male could not be used for the social novelty preference (calculation divided through 0) and one ko male was detected as outlier in the novelty object preference. Therefore, these animals were excluded from analysis.

Electrophysiology in primary mouse culture

Electrophysiological recordings in the whole-cell voltage-clamp mode was performed using an EPC-10 patch-clamp amplifier and PULSE software (HEKA Elektronik, Lambrecht, Germany) from in vitro mouse hippocampal culture at DIV 8-10 visualized with a charge-coupled-device (CCD) camera (VX55, TILL Photonics GmbH, Gräfelfing, Germany) mounted on an upright microscope (BX51WI, Olympus, Hamburg, Germany). Genotypes were equally distributed across the days. Coverslips with transfected cells were transferred at room temperature with a bath solution containing 156 mM NaCl, 2 mM KCl, 2 mM CaCl 2, 1 mM MgCl2, 16.5 mM glucose, and 10 mM HEPES (pH 7.3 with NaOH) to which PTX (50 µM) and tetrodotoxin (0.5 µM) were added during recording. The pipette solution contained 110 mM CsMeSO3, 25 mM CsCl, 30 mM HEPES,2 mM MgCl2, 0.362 mM CaCl2, 1 mM EGTA, 4 mM MgATP, and 0.1 mM Na2GTP (pH7.2 with CsOH). Patch pipettes were pulled from borosilicate glass (Science Products, Hofheim, Germany) and had resistances of 3 to 7 MΩ when filled with the pipette solution. Neurons were held at a potential of −70 mV, and mEPSCs were analyzed for 100 s current recordings made after 10 min of equilibration in the whole-cell configuration. Data were acquired at a sampling rate of 20 kHz and filtered at 3 kHz. Series resistance was controlled every 5 min, and only experiments with uncompensated series resistances of <25 MΩ were accepted. Mean event

amplitude and frequency were determined off-line with the Mini Analysis program (Synaptosoft Inc.) using an amplitude threshold of −5 pA. The inter-event interval was calculated through the software Mini Analysis putting the bin to 1 ms. We considered the cumulative distribution of the events in the range from 1 ms to 3 s interval. Please note that the average of inter-event interval that were showed has time binning intervals of 100 ms. For the inter-event calculation two cell conditions (one wt and one ko) were taken out due to technical data problems.

Cell culture, transfection and stimulation

Primary cultures of miR379-410 mice hippocampal neurons from P1 pups were performed, using the same procedure as for rat culture, except from following changes. Each hippocampus was dissected and later collected in Leibovitz`s L15 medium (Life Technologies, Carlsbad, CA, USA) with 7 mM HEPES (L15+H) in a 1.5 ml reaction tube. Afterwards, L15+H medium was carefully removed and 500µl of TrypLE Express was added for 7 min at 37°C by gently inversing the tube every minute for three times. After the dissociation step of hippocampi cells in NB+ medium, cells were plated on poly L-lysine coated coverslips and after 5 h of plating, medium were removed completely and fresh NB+ medium will be added to the cells. Neuronal transfections were performed with Lipofectamine 2000 (Thermo Fisher Scientific, Darmstadt, Germany) as described earlier[2]. For stimulation, 18 DIV neurons were treated with Picrotoxin (PTX; 100 µM final concentration; Merck, Darmstadt, Germany) or solvent (ethanol absolute) as vehicle control for the indicated times. Cells were transfected with respective Anti-miRs (pLNAs, Exiqon, Vedbaek, Denmark) or miRNA mimics premiR miRNA precursor Ambion, Thermo Fisher Scientific, Darmstadt, Germany).

Western blot

For each lane, 15 µg protein were run on a 4-20% Bio-Rad precast gradient gel (Bio-Rad, Dreieich, Germany) next to the Precision Plus Protein Dual Color Standard (Bio-Rad, Dreieich, Germany) in SDS-PAGE running buffer and transferred to a methanol-activated Amersham Hybond PVDF (poly-vinylidene difluoride) membrane (GE Healthcare, Freiburg im Breisgau) by tank blotting at 90 V for 90 min in a cold room (4-6 °C) in blotting buffer. The membrane was blocked for 2 h in TBS-T (tris buffered

saline supplemented with Tween) containing 5 % milk powder. Antibody dilutions (anti-Cnih2 (1:1000 polyclonal rabbit; Synaptic Systems), anti-Prr7 (TRAP3/10) (1:500 monoclonal mouse; Thermo Fisher Scientific), and anti-alpha-tubulin (1:2000 polyclonal rabbit; New England biolabs) were prepared in TBS-T/milk and membrane were incubated overnight at 4°C under slight shaking. After three washes with TBS-T/milk, the membrane was incubated with HRP (horseradish peroxidase)-conjugated secondary antibodies in TBS-T/milk for 1.5 h under slight shaking. Then membranes were washed three times with TBS-T before developing with Biorad ECL reagent (Bio-Rad, Dreieich, Germany) for 5 min in the dark. Signals were detected using biorad gel doc imager (Bio-Rad, Dreieich, Germany).

Genotyping of the miR379-410 and Thy1-GFP mice

Before weaning, mice were marked and genotyped at Pnd21 with the Kapa Mouse Genotyping kit (Merck, Darmstadt, Germany) by using earmark tissues. miR379-410 animals were genotyped with the primers indicated in Appendix Figure S1 and validated as described previously in more details[1]. The Thy1GFP reporter mouse line was cross-bred with the miR379-410 line for spine morphology analysis.

Primer sequences

Cloning:

Prr7_UTR_Fwd: AAACTCGAGAGGACTACAGCCGTATAGAGG

Prr7_UTR_Rev: TTTGTCGACGTACCAAAGCAGATCACACACC

Src_UTR_Fwd: AAACTCGAGCTCTCTGGAGTTAGCCTGCTTC

Src_UTR_Rev: TTTGTCGACATGGACACAAGGGAAGACACACAG

Cnih2_UTR_Fwd: AAACTCGAGAGTATGGTTTATACGTTGGTGAGCTTC

Cnih2_UTR_Rev: TTTGTCGACGCTGGACTCCTCCAGGCAAC

Dlgap3_UTR_Fwd: AAACTCGAGCCAGACCAGGCTGTGACC

Dlgap3_UTR_Rev: TTTGTCGACTTCCGGTGCAGTTCTGCGG

Mutagenesis:

Prr7_mut1_Fwd: TACCCTGTTGAATTCATTTTGAGGATAATAAAGG

Prr7_mut1_Rev: TCCTCAAAATGAATTCAACAGGGTAAGAAATCC

Prr7_mut2_Fwd: ATAATAAAGGTCTAGAATCTGCTTTGGTACGtCG
Prr7_mut2_Rev: ACCAAAGCAGATTCTAGACCTTTATTATCCTCAAAATG
Src_mut_Fwd: GGTGGTTTTCCATCTAGGACCCACTGCGCTCACCTGG
Src_mut_Rev: CCAGGTGAGCGCAGTGGGTCCTAGATGGAAAACCACC
Cnih2_mut_Fwd: GCACTGGTGCCTCCCGGGTCTCCACCCCCCAAACTGCTG
Cnih2_mut_Rev: CAGCAGTTTGGGGGGTGGAGACCCGGGAGGCACCAGTGC
Dlgap3_mut_Fwd: ACCTGTGGCTGTTCTAGAATCCCTTTGAGTATCCCAG
Dlgap3_mut_Rev: TACTCAAAGGGATTCTAGAACAGCCACAGGTGTGGTGAGG

qPCR:
pre-miR-124a fw: CGTGTTCACAGCGGACCTT
pre-miR-124a rev: TCACCGCGTGCCTTAATTG
pre-miR-132 fw: ACCGTGGCTTTCGATTGTTAC
pre-miR-132 rev: CGACCATGGCTGTAGACTGTTAC
pre-miR-134 fw: TGTGACTGGTTGACCAGAGGG
pre-miR-134 rev: GGTGACTAGGTGGCCCACAG
pre-miR-137 fw: GGTGACGGGTATTCTTGGGT
pre-miR-137 rev: CGACTACGCGTATTCTTAAGCAAT
pre-miR-138.2 fw: AGCTGGTGTTGTGAATCAGGC
pre-miR-138.2 rev: GTGAAATAGCCGGGTAAGAGGAT
Cnih2 fw: CCTCCCTCATCTTCTTTGTCATC
Cnih2 rev: GTACCTCCAGAGGTGGTAGAA
Dlgap3 fw: AGCAGTACCTTCCCCAGGAT
Dlgap3 rev: AAACTGGTCCAGGAGTGTGG
Lzts2 fw: GCAGCGTGAGCAGTCTTATCT
Lzts2 rev: AGGTAAAGCTATTGCCTGGGA
Mpp2 fw: GCCACGAACTCCGAGTCTG
Mpp2 rev: GCCTCGAAGAAAGATCAGGTC
RP2 fw: CCACAGAAGCCAATAGAAGCA
RP2 rev: GGGAGAAGCCTTTACCAACC
Prr7 fw: GCTTTCGTTGCCACTGTCTG
Prr7 rev: TTCGAACTCGTCTTCCTGCC
Prr12 fw: GCGGGATGGAGTTACGAGAG
Prr12 rev: CGATGCAAGATGTCGGTTTCT

U6 fw: CTCGCTTCGGCAGCACA
U6 rev: AACGCTTCACGAATTTGCGT
Shank1 fw: AGCCTGCAGCAGTGCCCAGCA
Shank1 rev: ATGCGAGGCCGCCAGGCCCA
Shank3 fw: TGGTTGGCAAGAGATCCAT
Shank3 rev: TTGGCCCCATAGAACAAAAG
Shb fw: CTGATGACTACTCCGATCCCTT
Shb rev: GGGGTGTCGTACAACTGGATG
Src fw: TACGAGGCCCAAAAGATGATGG
Src rev: GTGTCATAGAGAGGTAGGGGTT
Ywhaz fw: TGGAAGTCCTGCCCTAAATG
Ywhaz rev: GAGGAGGAGGAGGAGGAAGA

Genotyping:
169–379lox-fw: GCCACTGCTTACTCTCATCTGC
170–379lox-rev: CCGTATTATCCCATCAAGTAGC
171–410lox-fw: CCAGATGTGCAATGGATGG
173–410lox-rev: AAAGAGAGGTGACCATGCACTG
Thy1GFP-555: TCTGAGTGGCAAAGGACCTTAGG
Thy1GFP-556: CGCTGAACTTGTGGCCGTTTACG

Processing of RNAseq data

We removed sequencing adapter and quality-trimmed all short reads from the 3'end using FLEXBAR 2.5.3 and a Phred score cutoff >10. All reads longer than 18bp were retained and rRNA reads were subtracted *in silico* using bowtie2 and an index of the complete repeating rRNA unit (BK000964.3). We used the STAR aligner 2.4.2a[4] to map against the mouse genome (EnsEMBL 79 genome + annotations). We performed differential gene expression analysis using the Cuffdiff 2.2.1 and used TargetScan 7 to annotate miRNA binding sites in our gene set.

GO-Term Enrichment Analysis and Bipartite Network representation

Bioinformatic analysis were compiled using R-Studio (1.1.383) with R version 3.4.3. Bioconductor packages topGo (2.30.0) and org.Mm.eg.db (3.5.0) were used for the GO-Term enrichment analysis as described in the topGo script. The elim algorithm[5] with Fisher's exact test was employed to compare the enrichment of cellular component GO-Terms in the differentially expressed genes (DEG, 3068 genes) against the specific sequencing background (13975 genes). Along with the manual for this algorithm, we did not perform further multiple testing correction. Minimum nodeSize was set to 5, as suggested in the documentation. GO-Terms and associated genes were subsequently plotted as bipartite Terms-Genes network, using the FGNet (3.12.0) package[6]. The source code of the package was rewritten to use the elim algorithm of the topGo package together with Fisher's exact test to build a terms-genes incidence matrix as described in the FGNet documentation. The layout of the resulting bipartite network was modified with Cytoscape (3.4.0). Synapse associated GO-Terms and associated genes are enlarged in the final figure. GO-Terms with more than 300 annotated genes are not included in the plots.

String Database Protein Interaction Network

To perform hierarchical clustering (unweighted pair group method with arithmetic mean) of identified GO-Terms and associated Genes, the FGNet derived terms genes incidence matrix was converted into a binary distance matrix. The resulting Dendrogram was plotted together with the distance matrix using R packages ComplexHeatmap (1.17.1)[7], RColorBrewer (1.1-2) and circlize (0.4.3). Next, DEG associated with GO-Terms referring to synaptic function ("Chloride channel complex", "GABA-A receptor complex", "GABA receptor complex", "Synaptic membrane", "Postsynaptic membrane", "Ionotropic glutamate receptor complex" and "AMPA glutamate receptor complex") were used as input in the String Database (Version 10.5) to construct a protein interaction network. String-DB Settings were the following:

Meaning of network edges: evidence

Active interaction sources: textmining, experiments and databases

Minimum required interaction score: medium confidence

Max numbers of interactors to show: 1st shell (80 interactors), 2nd shell (20 interactors).The network layout was adapted with Cytoscape.

miRNA overrepresentation analysis

A list of all the transcripts containing predicted miR379-410 miRNAs was generated. First, mouse miRNAs and their conserved binding sites (8mer sites, 7mer-m8 sites and 7mer-1a sites) were extracted from the file "Summary Counts, all predictions" (Targetscan 7.1[8]) using the R package data.table (1.10.4-3). Subsequently miRNA names were used to filter for transcripts containing miR379-410 Cluster miRNA binding sites (see Suppl. Table 1 for the list of names). mmu-miR-1962, which shares the same seed as mmu-miR-485-5p, was not included in the analysis. Next, each transcript from obtained list was compared to its log2-fold change in knockout animals determined by RNA-seq (see RNAseq and bioinformatics analysis). This allowed summing up miRNA binding sites in significantly up- and downregulated transcripts with Microsoft Excel 2016. miRNAs with less than 5 total binding sites in differentially expressed transcripts were excluded. To enhance specificity in the analysis, miRNAs with an expression value (RPM) of less than 100 in the mouse brain (values obtained from[9]) were filtered out. The plot was compiled with the R package plotly (4.7.1).

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Appendix: Two-way ANOVAs with genotype and sex between-subject factors

Fig.1a (left panel): Isolation-induced USV in Pups (total call number)
Genotype: $F_{1,29}$=4.979, p=0.034
Sex: $F_{1,29}$=0.989, p=0.328
Genotype x Sex: $F_{1,29}$=0.696, p=0.411

Fig.1a (right panel): Isolation-induced USV in pups (rm-ANOVA, total call number – developmental course)
Development: $F_{3,87}$=25.463, p<0.001
Genotype: $F_{1,29}$=4.979, p=0.034
Sex: $F_{1,29}$=0.989, p=0.328
Development x Genotype: $F_{3,87}$=1.403, p=0.247
Development x Sex: $F_{3,87}$=3.384, p=0.022
Development x Genotype x Sex: $F_{3,87}$=0.143, p=0.934
Genotype x Sex: $F_{1,29}$=0.696, p=0.411

Fig.1b (right panel): Reciprocal Social Interaction (interaction time)
Genotype: $F_{1,47}$=8.592, p=0.005
Sex: $F_{1,47}$<0.001, p=0.990
Genotype x Sex: $F_{1,47}$=0.562, p=0.457

Fig.1c (right panel): Reciprocal Social Interaction (emitted USV)
Genotype: $F_{1,47}$=14.628, p<0.001
Sex: $F_{1,47}$=0.948, p=0.335
Genotype x Sex: $F_{1,47}$=0.008, p=0.928

Fig.1d (right panel): Three-chamber social approach (social preference subject/object)
Genotype: $F_{1,105}$=4.923, p=0.029
Sex: $F_{1,105}$=0.154, p=0.695
Genotype x Sex: $F_{1,105}$=0.783, p=0.378

Appendix Fig.S3a: Three-chamber social approach (rm-ANOVA, time spent sniffing)
Side: $F_{1,105}$=150.515, p<0.001
Genotype: $F_{1,105}$=0.682, p=0.411
Sex: $F_{1,105}$=9.285, p=0.003
Side x Genotype: $F_{1,105}$=1.541, p=0.217
Side x Sex: $F_{1,105}$=0.743, p=0.391
Side x Genotype x Sex: $F_{1,105}$=0.925, p=0.338
Genotype x Sex: $F_{1,105}$=0.101, p=0.751

Appendix Fig.S3b (left panel): Three-chamber social recognition (rm-ANOVA, time spent sniffing)
Side: $F_{1,105}$=27.247, p<0.001
Genotype: $F_{1,105}$=9.172, p=0.003
Sex: $F_{1,105}$=12.465, p=0.001
Side x Genotype: $F_{1,105}$=0.056, p=0.814
Side x Sex: $F_{1,105}$=0.001, p=0.972
Side x Genotype x Sex: $F_{1,105}$=0.518, p=0.473
Genotype x Sex: $F_{1,105}$=0.572, p=0.451

Appendix Fig.S3b (right panel): Three-chamber social recognition (novelty preference novel/familiar)
Genotype: $F_{1,104}$=0.173, p=0.679
Sex: $F_{1,104}$=1.773, p=0.186
Genotype x Sex: $F_{1,104}$=0.598, p=0.441

Appendix Fig.S3c (left panel): Three-chamber object acquisition (rm-ANOVA, time spent sniffing)
Side: $F_{1,106}$=2.649, p=0.107
Genotype: $F_{1,106}$=1.459, p=0.230
Sex: $F_{1,106}$=0.472, p=0.493
Side x Genotype: $F_{1,106}$=0.149, p=0.700
Side x Sex: $F_{1,106}$=1.896, p=0.171
Side x Genotype x Sex: $F_{1,106}$=0.470, p=0.494
Genotype x Sex: $F_{1,106}$=2.002, p=0.160

Appendix Fig.S3c (right panel): Three-chamber object acquisition (object preference object1/object2)

Genotype: $F_{1,106}$=0.466, p=0.496

Sex: $F_{1,106}$=2.041, p=0.156

Genotype x Sex: $F_{1,106}$=0.008, p=0.927

Appendix Fig.S3d (left panel): Three-chamber object recognition (rm-ANOVA, time spent sniffing)

Side: $F_{1,106}$=12.740, p=0.001

Genotype: $F_{1,106}$=0.008, p=0.930

Sex: $F_{1,106}$=3.377, p=0.069

Side x Genotype: $F_{1,106}$=0.359, p=0.550

Side x Sex: $F_{1,106}$=0.700, p=0.405

Side x Genotype x Sex: $F_{1,106}$=0.101, p=0.751

Genotype x Sex: $F_{1,106}$=2.730, p=0.101

Appendix Fig.S3d (right panel): Three-chamber object recognition (novelty preference novel/familiar)

Genotype: $F_{1,106}$=1.202, p=0.275

Sex: $F_{1,106}$=0.072, p=0.789

Genotype x Sex: $F_{1,106}$=0.092, p=0.762

Fig.EV1a: Open Field (time spent in center)

Genotype: $F_{1,100}$=8.868, p=0.004

Sex: $F_{1,100}$=0.158, p=0.692

Genotype x Sex: $F_{1,100}$=0.028, p=0.867

Fig.EV1d: Elevated Plus Maze (time spent in open arms %)

Genotype: $F_{1,100}$=9.987, p=0.002

Sex: $F_{1,100}$=0.876, p=0.352

Genotype x Sex: $F_{1,100}$=5.551, p=0.020

When splitting the data by sex, the genotype effect is mainly in females ($p<0.001$) but not in males (p=0.5905).

Fig.EV1e: Elevated Plus Maze (number of entries into open arms)

Genotype: $F_{1,100}$=21.699, p<0.001

Sex: $F_{1,100}$=3.003, p=0.086

Genotype x Sex: $F_{1,100}$=1.333, p=0.251

Fig.EV1f: Marble Burying (half + completely buried marbles)

Genotype: $F_{1,98}$=6.094, p=0.015

Sex: $F_{1,98}$=1.000, p=0.320

Genotype x Sex: $F_{1,98}$=2.795, p=0.098

Fig.EV1g: Homing Test (PND9)

Genotype: $F_{1,24}$=1.221, p=0.280

Sex: $F_{1,24}$=5.288, p=0.030

Genotype x Sex: $F_{1,24}$=0.135, p=0.716