Expanded View Figures

Figure EV1. miR379-410 ko mice demonstrated increased anxiety-related behaviour.

A Open field test in adult mice. Time spent in the central arena was examined, wt $n = 51$ (male $n = 24$ and female $n = 27$), ko $n = 53$ (male $n = 24$ and female $n = 29$), $t_{102} = 3.020$, $^{**}P = 0.0032$, unpaired Student's t-test.

B–E Elevated plus maze test in adult mice. (B) Time spent on open arms (percentage) are shown, wt $n = 24$ (male), ko $n = 24$ (male), $t_{46} = 0.5419$, ns $P = 0.5905$. unpaired Student's t-test. (C) Time spent on open arms (percentage) is shown, wt $n = 27$ (female), ko $n = 29$ (female), $t_{54} = 4.099$, $^{***}P < 0.001$, unpaired Student's t-test. (D) Time spent on open arms (percentage) is shown for pooled data of male and female; wt $n = 51$ (male $n = 24$ and female $n = 27$), ko $n = 53$ (male $n = 24$ and female $n = 29$), $t_{102} = 3.265$, $^{**}P = 0.0015$, unpaired Student's t-test. Please notice that the genotype effect is mainly in females ($P < 0.001$) but not in males ($P = 0.5905$); Genotype × Sex: $F_{1,100} = 5.551$, $P = 0.020$, ANOVA. (E) Number of open arm entries is shown; wt $n = 51$ (male $n = 24$ and female $n = 27$), ko $n = 53$ (male $n = 24$ and female $n = 29$), $t_{102} = 4.679$, $^{****}P < 0.0001$, unpaired Student's t-test.

F Marble burying test as a measure for repetitive behaviour. Total number of marbles (half and completely) buried by adult miR379-410 wt and ko mice is shown; wt $n = 49$ (male $n = 23$ and female $n = 26$), ko $n = 53$ (male $n = 24$ and female $n = 29$), $t_{100} = 2.569$, $^{*}P = 0.0117$, unpaired Student's t-test.

G Pup homing test performed at P9 during developmental milestones. Time spent in the nest area (containing nest litter) is shown, wt $n = 17$ (male $n = 8$ and female $n = 9$); ko $n = 11$ (male $n = 5$ and female $n = 6$), $t_{26} = 0.9834$, ns $P = 0.3345$, unpaired Student's t-test.

Data information: Data are presented as mean ± s.e.m.

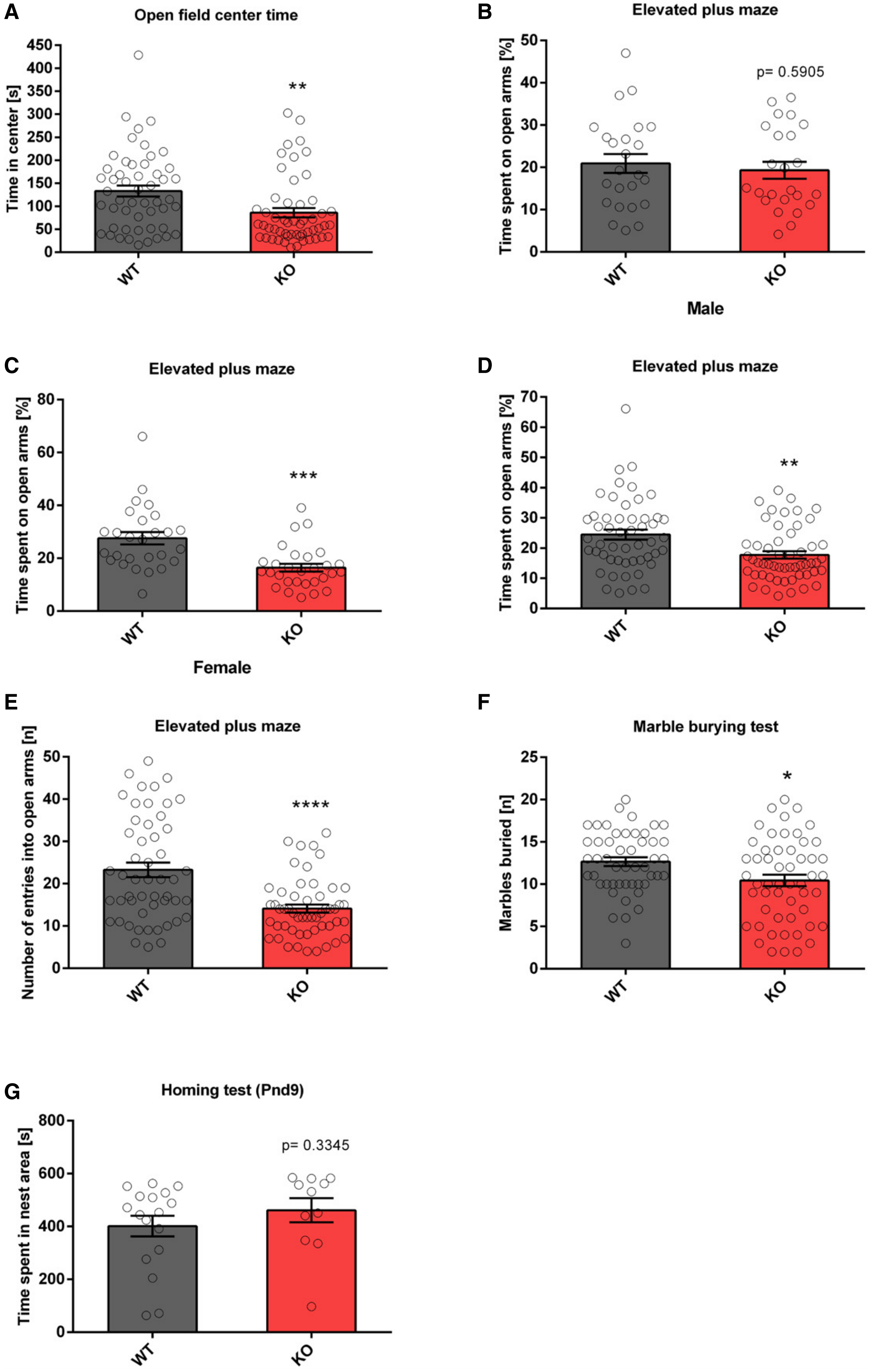

A
Open field center time
Time in center [s]
450
400
350
300
250
200
150
100
50
0
**
WT
KO
B
Elevated plus maze
Time spent on open arms [%]
50
40
30
20
10
0
p= 0.5905
WT
KO
Male
C
Elevated plus maze
Time spent on open arms [%]
80
60
40
20
0
***
WT
KO
Female
D
Elevated plus maze
Time spent on open arms [%]
70
60
50
40
30
20
10
0
**
WT
KO
E
Elevated plus maze
Number of entries into open arms [n]
50
40
30
20
10
0
****
WT
KO
F
Marble burying test
Marbles buried [n]
25
20
15
10
5
0
*
WT
KO
G
Homing test (Pnd9)
Time spent in nest area [s]
800
600
400
200
0
p= 0.3345
WT
KO

Figure EV1.

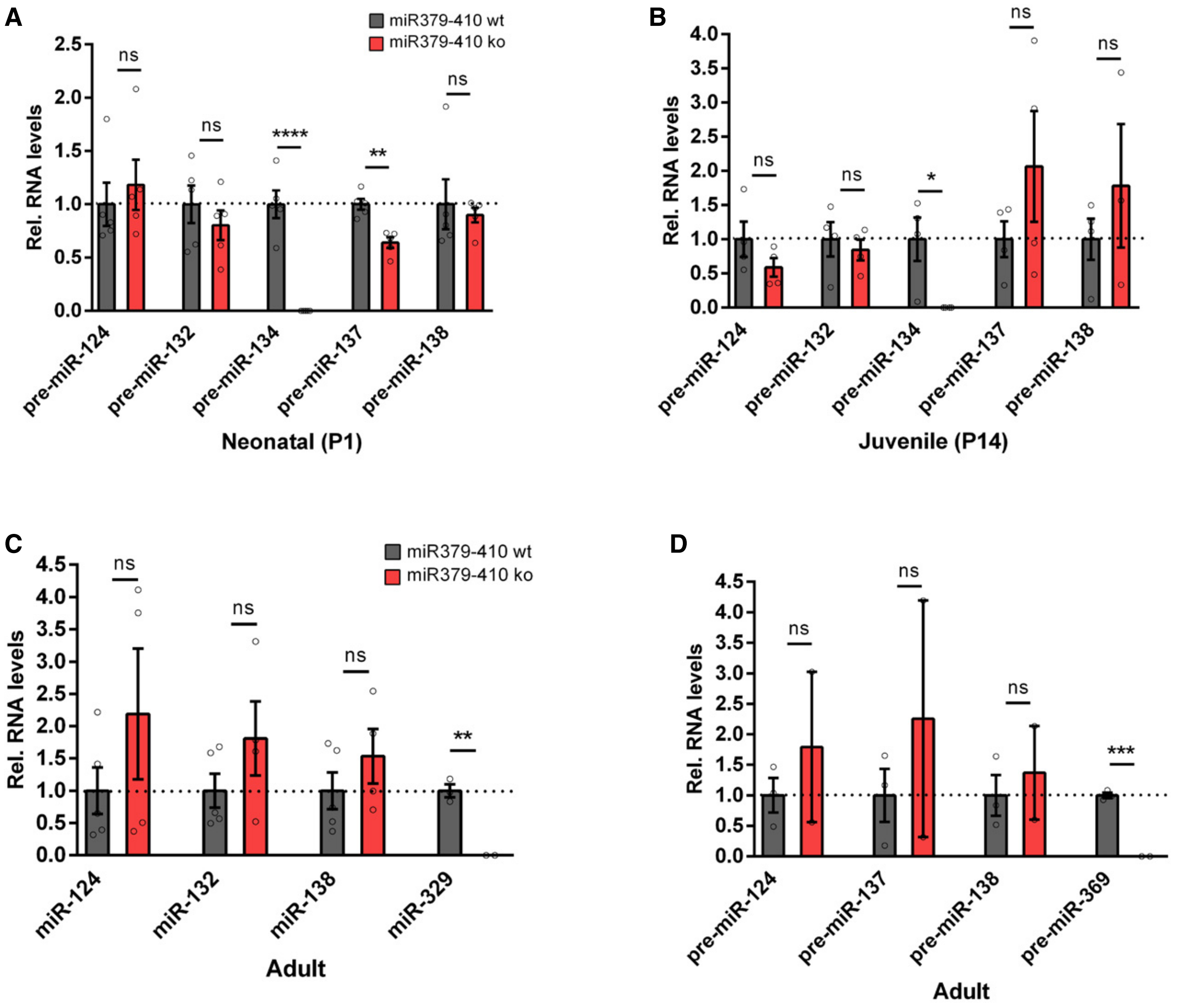


Figure EV2. qPCR expression levels of RNA samples in different brain regions and ages of miR379-410 wt/ko mice.

- A Expression of precursor miRNAs in hippocampal neonatal (P1) miR379-410 wt/ko pups: pre-miR-124 (wt *n* = 5, ko *n* = 5, t_8 = 0.5848, ns *P* = 0.5748), pre-miR-132 (wt *n* = 5, ko *n* = 5, t_8 = 0.8740, ns *P* = 0.4076), pre-miR-137 (wt *n* = 5, ko *n* = 5, t_8 = 5.037, ***P* = 0.0010), pre-miR-138.2 (wt *n* = 5, ko *n* = 5, t_8 = 0.4145, ns *P* = 0.6894) and from the miR379-410 cluster pre-miR-134 (wt *n* = 5, ko *n* = 5, t_8 = 7.637, *****P* < 0.0001), unpaired Student's *t*-test.
- B Expression of precursor miRNAs in hippocampal juvenile (P14) miR379-410 wt/ko mice: pre-miR-124 (wt *n* = 4, ko *n* = 4, t_6 = 1.406, ns *P* = 0.2093), pre-miR-132 (wt *n* = 4, ko *n* = 4, t_6 = 0.5348, ns *P* = 0.6120), pre-miR-137 (wt *n* = 4, ko *n* = 4, t_6 = 1.250, ns *P* = 0.2578), pre-miR-138.2 (wt *n* = 4, ko *n* = 3, t_5 = 0.9339, ns *P* = 0.3932) and from the miR379-410 cluster pre-miR-134 (wt *n* = 4, ko *n* = 4, t_6 = 3.150, **P* = 0.0198); unpaired Student's *t*-test.
- C Expression of mature miRNAs in cerebellum of adult miR379-410 wt/ko mice: miR-124 (wt *n* = 5, ko *n* = 4, t_7 = 1.214, ns *P* = 0.2642), miR-132 (wt *n* = 5, ko *n* = 4, t_7 = 1.382, ns *P* = 0.2095), miR-138 (wt *n* = 5, ko *n* = 4, t_7 = 1.089, ns *P* = 0.3120) and from the miR379-410 cluster miR-329 (wt *n* = 3, ko *n* = 2, t_3 = 7.720, ***P* = 0.0045); unpaired Student's *t*-test.
- D Expression of precursor miRNAs in cerebellum of adult miR379-410 wt/ko mice: pre-miR-124 (wt *n* = 3, ko *n* = 2, t_3 = 0.8014, ns *P* = 0.4815), pre-miR-137 (wt *n* = 3, ko *n* = 2, t_3 = 0.8107, ns *P* = 0.4769), pre-miR-138.2 (wt *n* = 3, ko *n* = 2, t_3 = 0.5169, ns *P* = 0.6409) and from the miR379-410 cluster pre-miR-369 (wt *n* = 3, ko *n* = 2, t_3 = 16.78, ****P* = 0.0005); unpaired Student's *t*-test. Data are presented as mean ± s.e.m.

Figure EV3. Clustering of synaptic GO terms and String database protein–protein interaction network.

- A Binary distance matrix of detected GO terms (cellular component). Hierarchical clustering has been performed by the unweighted pair group method with arithmetic mean. GO terms with more than 300 annotated genes are not shown.
- B Complete String database protein–protein interaction network.

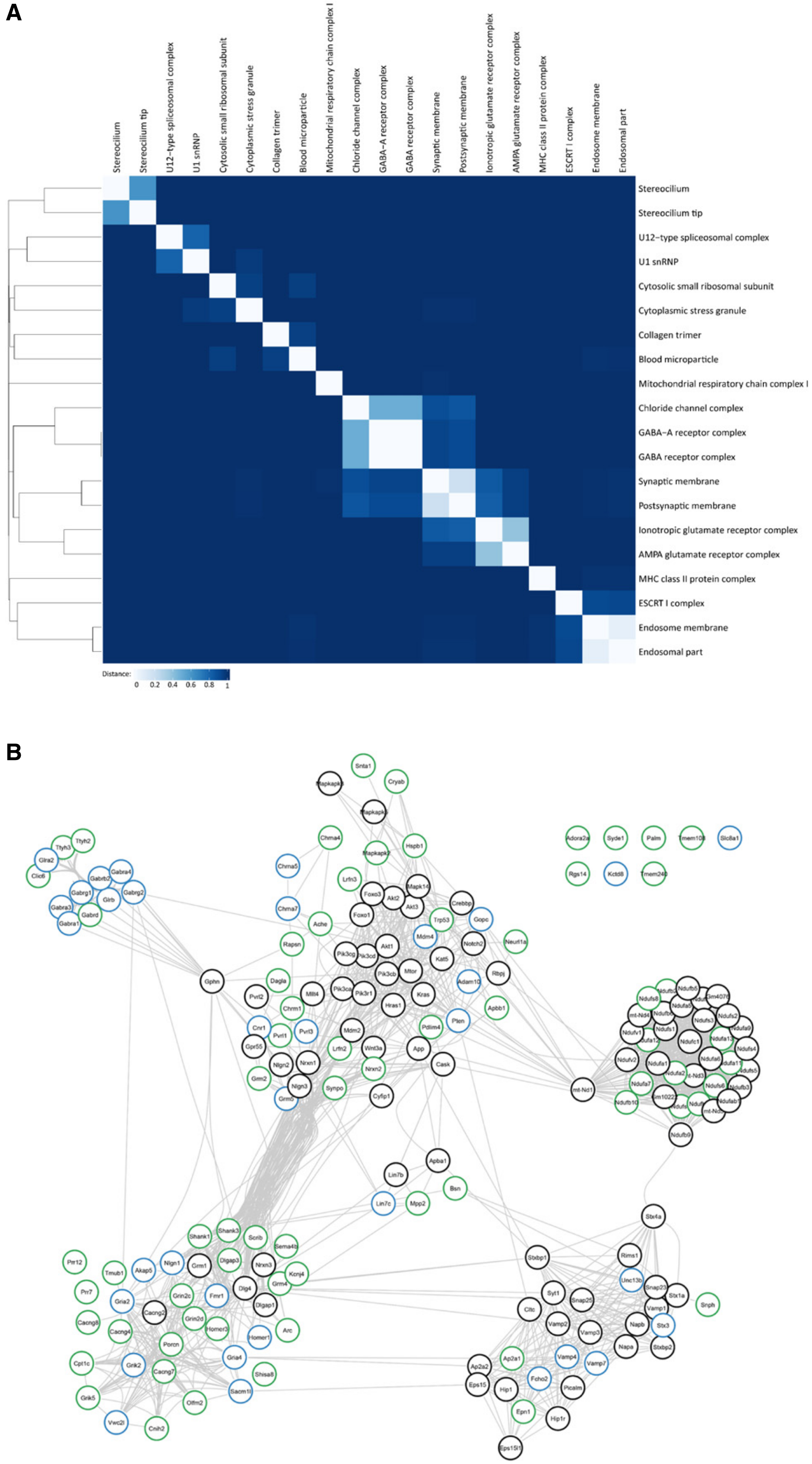
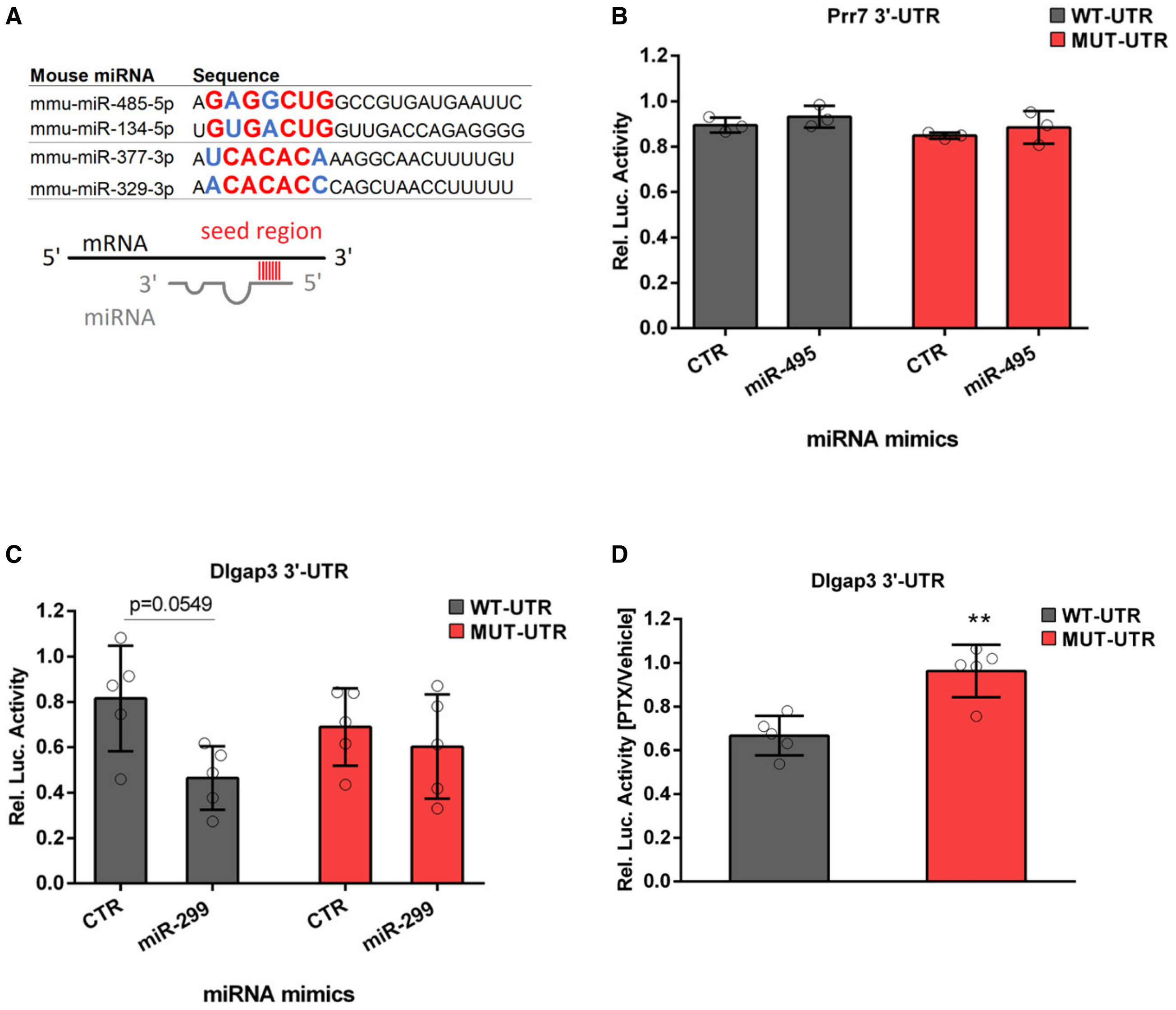

A
Stereocilium
Stereocilium tip
U12−type spliceosomal complex
U1 snRNP
Cytosolic small ribosomal subunit
Cytoplasmic stress granule
Collagen trimer
Blood microparticle
Mitochondrial respiratory chain complex I
Chloride channel complex
GABA−A receptor complex
GABA receptor complex
Synaptic membrane
Postsynaptic membrane
Ionotropic glutamate receptor complex
AMPA glutamate receptor complex
MHC class II protein complex
ESCRT I complex
Endosome membrane
Endosomal part
Distance:
0
0.2
0.4
0.6
0.8
1
B
Snta1
Cryab
Mapkapk3
Mapkapk5
Chrna4
Mapkapk2
Hspb1
Adora2a
Syde1
Palm
Tmem108
Slc8a1
Rgs14
Kctd8
Tmem240
Ttyh2
Ttyh3
Glra2
Clic6
Gabrb2
Gabra4
Gabrg1
Glrb
Gabrg2
Gabra3
Gabrd
Gabra1
Chrna5
Lrfn3
Foxo3
Akt2
Mapk14
Akt3
Crebbp
Chrna7
Ache
Foxo1
Trp53
Gopc
Mdm4
Notch2
Neurl1a
Rapsn
Akt1
Pik3cg
Pik3cd
Kat5
Gphn
Pik3cb
Mtor
Adam10
Rbpj
Dagla
Pvrl2
Mllt4
Pik3ca
Pik3r1
Kras
Apbb1
Chrm1
Hras1
Cnr1
Pvrl1
Pvrl3
Mdm2
Pdlim4
Pten
Gpr55
Lrfn2
Wnt3a
App
Nlgn2
Nrxn1
Nrxn2
Cask
Grm2
Nlgn3
Synpo
Cyfip1
Grm5
mt-Nd1
Apba1
Lin7b
Bsn
Lin7c
Mpp2
Stx4a
Shank1
Shank3
Scrib
Sema4b
Prr12
Tmub1
Akap5
Nlgn1
Grm1
Dlgap3
Nrxn3
Kcnj4
Stxbp1
Rims1
Prr7
Gria2
Grin2c
Fmr1
Dlg4
Grm4
Unc13b
Snap23
Stx1a
Snph
Cacng2
Dlgap1
Syt1
Snap25
Cacng8
Grin2d
Cltc
Vamp1
Cacng4
Homer2
Homer1
Arc
Vamp2
Vamp3
Napb
Stx3
Porcn
Napa
Stxbp2
Cpt1c
Grik2
Gria4
Cacng7
Ap2a2
Ap2a1
Vamp4
Vamp7
Shisa8
Eps15
Hip1
Fcho2
Picalm
Sacm1l
Olfm2
Grik5
Epn1
Hip1r
Vwc2l
Cnih2
Eps15l1
Ndufs8
Ndufb5
Gm4076
mt-Nd4
Ndufs2
Ndufa9
Ndufv1
Ndufs1
Ndufs3
Ndufa12
Ndufc1
Ndufa13
Ndufs4
Ndufv2
Ndufa1
Ndufa6
Ndufa11
Ndufs5
Ndufa2
mt-Nd3
Ndufa7
Ndufs6
Ndufb3
Ndufb10
Gm10221
Ndufab1
mt-Nd5
Ndufb9

Figure EV3.



Mouse miRNA	Sequence
mmu-miR-485-5p	AGAGGCUGGCCGUGAUGAAUUC
mmu-miR-134-5p	UGUGACUGGUUGACCAGAGGGG
mmu-miR-377-3p	AUCACACAAAGGCAACUUUUGU
mmu-miR-329-3p	AACACACCCAGCUAACCUUUUU

Figure EV4. miRNA binding site analysis and target validation.

A miRNAs of the miR379-410 cluster sharing similar seed regions: miR485-5p/miR-134-5p and miR-377-3p/miR-329-3p. Seed regions (nucleotide 2–8) are highlighted in bigger letters, conserved nucleotides in red and those that allow wobble base pairing in blue.

B 3′UTR luciferase reporter gene assays in cultured rat cortical neurons using the Prr7 3′UTR reporter gene (wt or seed mutant) together with miR-495 or control mimic, respectively (n = 3 per group; wt: P = 0.7775; mut: P = 0.7797; mimic: $F_{1,8}$ = 1.816, P = 0.2147; utr-construct: $F_{1,8}$ = 2.973, P = 0.1229; mimic × utr-construct: $F_{1,8}$ = 9.47e-006, P = 0.09976); two-way ANOVA.

C 3′UTR luciferase reporter gene assays in cultured rat cortical neurons using the Dlgap3 3′UTR reporter gene (wt or seed mutant) together with miR-299 or control mimic, respectively (n = 5 per group; wt: P = 0.0549; mut: P = 0.8982; mimic: $F_{1,16}$ = 6.145, P = 0.0247; utr-construct: $F_{1,16}$ = 0.005108, P = 0.9434; mimic × utr-construct: $F_{1,16}$ = 2.246, P = 0.1534); two-way ANOVA.

D 3′UTR luciferase reporter gene assays in cultured rat hippocampal neurons using the Dlgap3 3′UTR reporter gene (wt or seed mutant) after 48 h PTX stimulation (n = 5 per group, t_8 = 4.397, $^{**}P$ = 0.0023); unpaired Student's t-test.

Data information: Data are presented as mean ± s.d.

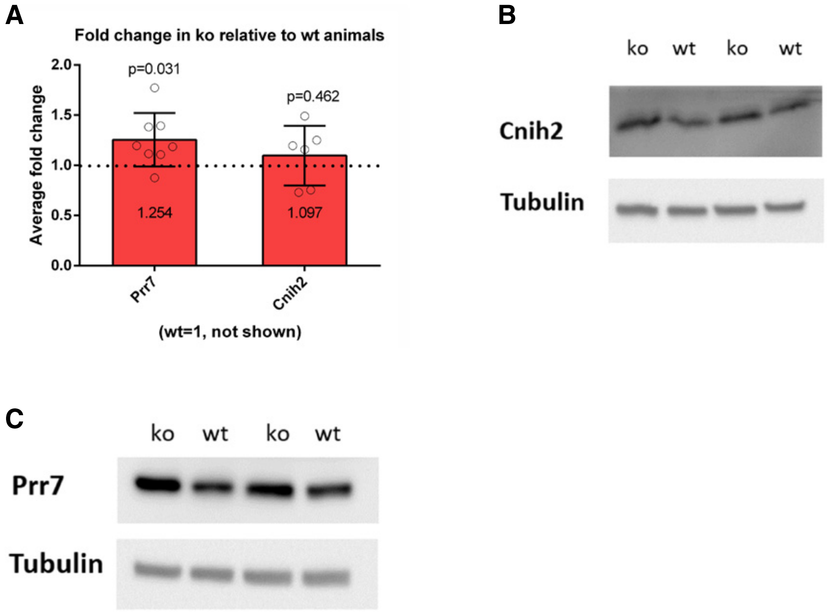


Figure EV5. Protein expression in hippocampus of 5-week-old juvenile miR379-410 wt and ko mice.

A Average fold change in ko relative to wt animals is presented (wt = 1, not shown), Cnih2 (wt *n* = 6, ko *n* = 6, *P* = 0.4625), Prr7 (wt *n* = 7, ko = 8, *P* = 0.0311), one-sample *t*-test (2.3 test in excel). Data are presented as mean ± s.d.

B, C Representative Western blots of Cnih2 and Prr7 protein expression in hippocampus of juvenile mice.