Figure S1. Synaptosome RT-qPCR threshold cycle (Ct) values (to accompany Figure 2).
Table showing the Ct values from comparative RT-qPCR performed on RNA extracted from total and synaptosome fractions of mouse forebrain. The Ct values were used to determine the concentrations of RNA in the synaptosome fraction relative to the concentrations in the total homogenate (S:T).

Figure S2. Group data of puncta distribution.
(A) Pairwise comparisons of average puncta distance shown in Figure 3B.

(B) Fraction of puncta present in arbitrary bins. The 0-10 μm bin corresponds to the soma. Bins beyond 10 μm are in the dendrite. Error bars show standard deviation.
(C) Inverse cumulative distribution of puncta in arbitrary bins.
(D) Table to accompany Figure 3C. Left, fraction of puncta in each of the three subcompartments – soma, proximal, and non-proximal dendrites. Right, pairwise comparisons between genes in each subcompartment. Comparisons that are significantly different are in bold.

Figure S3. Controls for mRNA FISH
(A) GluA2 sense probe does not bind non-specifically. Red, GluA2 mRNA sense probe; cyan, MAP2; blue, Hoechst. Scale bar = 20 μm.
(B) Fads3 sense probe does not bind non-specifically. Scale bar = 20 μm.
(C) The DapB negative control, which is not complementary to any miRNA sequences, does not bind non-specifically

Figure S4. GluA2 FISH on cultures with different levels of activity.
(A-D) Distribution of FISH puncta after activity treatments. Dissociated neurons were treated with tetrodotoxin (TTX; 1 μM) or bicucullin (BIC; 40 μM) for (B) 15 min, (C) 1 hr, and (A,D) 3 hr. Results are plotted as an inverse cumulative distribution (i) and as proportions of puncta binned by cell compartment (ii). From each condition, 4 to 6 dendrites were quantified. Error bars show standard deviation. The distribution of miR-124 is included as a point of reference.
(E) Cultures treated with tetrodotoxin (TTX; 1 μM) or with bicucullin (BIC; 40 μM) for 3 hours before processing for FISH. Red, GluA2 or c-Fos mRNA; cyan, MAP2; blue, Hoechst. Scale bar = 20 μm.

Figure S5. Neuronal transduction efficiency of miR-124 overexpression and control lentiviruses.
Live images of transduced cultures taken before harvesting for protein. Co-expression of copGFP identifies transduced cells. Scale bar = 50 μm.

Figure S6. Sponge transductions.
(A) Sponge-124 and control sponge-CXCR lentiviral constructs.
(B) Western blot analysis of protein lysates from transduced cultures. Band intensities were quantified and normalized to Tuj1. The difference relative to control is shown below with standard error. N = 4 independent experiments.
(C) Live images of transduced cultures taken before harvesting for protein. Co-expression of copGFP identifies transduced cells. Scale bar = 50 μm.

Figure S7. Overexpression of miR-124 down-regulates the concentration of surface GluA2 at non-synaptic sites.

Measurement of non-synaptic GluA2 protein expression by surface labeling of GluA2 in live neurons followed by fixation and staining for synapsin. (i) Fraction of GluA2 puncta that are not apposed by synapsin puncta. (ii) Integrated intensity of GluA2 puncta that are not apposed synapsin puncta. N = 3 independent experiments, each with dendrites from 5 different neurons quantified. P values were determined by one-tailed t-tests. *P<0.05.

Figure S8: GluA2 FISH with digoxigenin-labeled riboprobes.

Digoxigenin-labeled riboprobes were generated from plasmids adapted with T7 or SP6 RNA polymerase sites and used for in situ hybridization on dissociated hippocampal neurons as described in Poon et al. (2006). Cells were incubated with HRP-conjugated DIG antibody and then processed for tyramide signal amplification using a Cy3-TSA kit (PerkinElmer). Blue, DAPI nuclear counterstain; red, GluA2 riboprobe. DIC: diffusion interference contrast image to show position of neuron with blue and red channels merged.

Figure S9: Effect of activity on miR-124 levels.

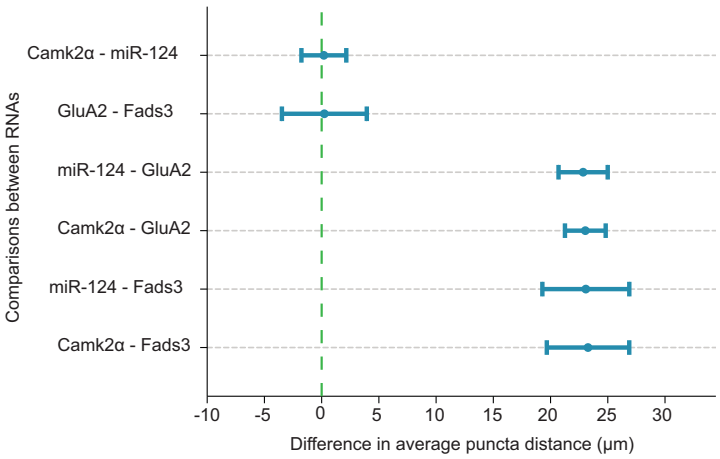
Cultured neurons were treated with tetrodotoxin (TTX; 1 μM) or bicuculline (BIC; 40 μM) and forskolin (FSK; 25 μM). RNA was extracted and used for RT-qPCR. (A) Table showing threshold cycle (Ct) values and fold-changes relative to the basal condition. (B) Graphical representation of fold-changes. Differences are not significant as determined by one-way analysis of variance (ANOVA). Error bars show S.E.M.

Figure S1.

Synaptosome prep		Camk2α	GluA2	Fads	pre-miR-124	miR-124	miR-134
1	Synaptosome Ct	20.43			30.62	24.73	
	Total Ct	20.12			26.42	25.26	
	S:T	0.81			0.05	1.44	
2	Synaptosome Ct	18.77	20.36	30.53	30.75	24.69	27.02
	Total Ct	19.20	17.74	28.37	25.07	25.71	28.12
	S:T	1.34	0.16	0.22	0.02	2.03	2.14
3	Synaptosome Ct	21.31	25.65	32.06	32.02	24.73	26.85
	Total Ct	20.86	22.97	30.29	29.91	25.17	27.82
	S:T	0.73	0.16	0.29	0.23	1.36	1.96
4	Synaptosome Ct	21.44	26.77	30.83	31.46	25.14	26.32
	Total Ct	20.99	23.25	28.04	27.42	25.48	27.53
	S:T	0.73	0.09	0.14	0.06	1.26	2.32

Figure S2.

A



D

Mean Puncta Proportion Observed in Soma Range -- [0, 10) Microns

Gene	Proportion
Camk2α	0.438
Fads3	0.799
GluA2	0.777
miR124	0.497

Gene 1	Gene 2	Difference	95% CI Limits		Pr > |t|
Camk2α	**Fads3**	**-0.361**	**-0.411**	**-0.311**	**< 0.001**
Camk2α	**GluA2**	**-0.339**	**-0.388**	**-0.290**	**< 0.001**
Camk2α	**miR124**	**-0.059**	**-0.107**	**-0.011**	**0.016**
Fads3	GluA2	0.022	-0.028	0.072	0.384
Fads3	**miR124**	**0.302**	**0.254**	**0.351**	**< 0.001**
GluA2	**miR124**	**0.280**	**0.232**	**0.328**	**< 0.001**

Mean Puncta Proportion Observed in Proximal Range -- [10, 30) Microns

Gene	Proportion
Camk2α	0.207
Fads3	0.129
GluA2	0.144
miR124	0.158

Gene 1	Gene 2	Difference	95% CI Limits		Pr > |t|
Camk2α	**Fads3**	**0.078**	**0.045**	**0.111**	**< 0.001**
Camk2α	**GluA2**	**0.063**	**0.031**	**0.095**	**< 0.001**
Camk2α	**miR124**	**0.049**	**0.018**	**0.081**	**0.002**
Fads3	GluA2	-0.015	-0.047	0.018	0.373
Fads3	miR124	-0.029	-0.060	0.003	0.078
GluA2	miR124	-0.014	-0.045	0.017	0.384

Mean Puncta Proportion Observed in Non-proximal Range -- [30, 150) Microns

Gene	Proportion
Camk2α	0.355
Fads3	0.072
GluA2	0.079
miR124	0.346

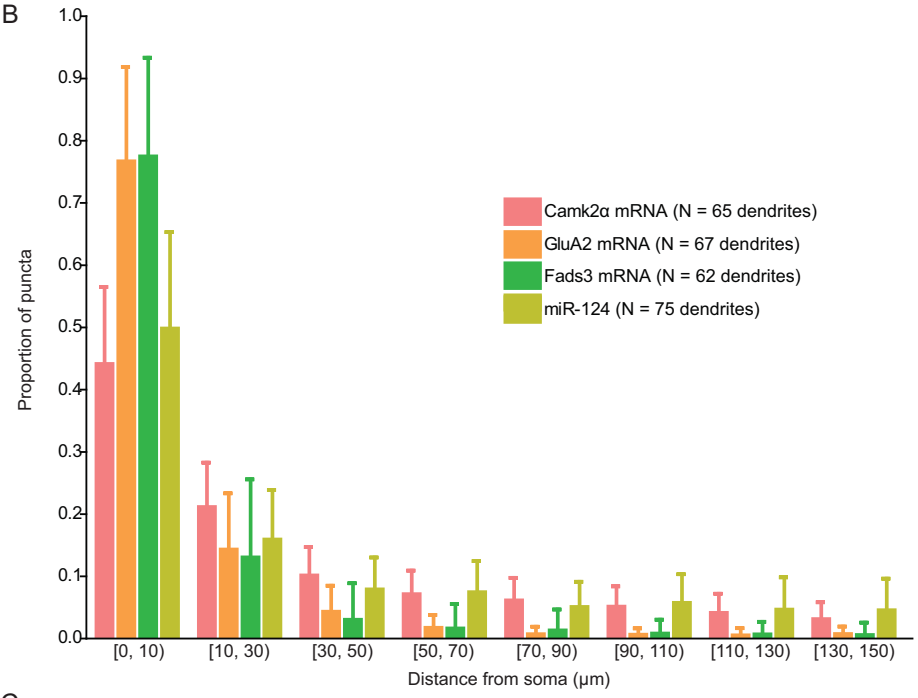
Gene 1	Gene 2	Difference	95% CI Limits		Pr > |t|
Camk2α	**Fads3**	**0.283**	**0.245**	**0.321**	**< 0.001**
Camk2α	**GluA2**	**0.276**	**0.239**	**0.313**	**< 0.001**
Camk2α	miR124	0.010	-0.026	0.046	0.600
Fads3	GluA2	-0.007	-0.045	0.030	0.702
Fads3	**miR124**	**-0.274**	**-0.310**	**-0.237**	**< 0.001**
GluA2	**miR124**	**-0.266**	**-0.302**	**-0.231**	**< 0.001**

Mean Puncta Distance from Cell Body (in Microns)

Gene	Distance
Camk2α	33.457
Fads3	10.184
GluA2	10.419
miR124	33.265

Gene 1	Gene 2	Difference	95% CI Limits		Pr > |t|
Camk2α	**Fads3**	**23.273**	**19.672**	**26.875**	**< 0.001**
Camk2α	**GluA2**	**23.038**	**21.258**	**24.818**	**< 0.001**
Camk2α	miR124	0.192	-1.768	2.152	0.848
Fads3	GluA2	-0.235	-3.944	3.473	0.901
Fads3	**miR124**	**-23.081**	**-26.879**	**-19.284**	**< 0.001**
GluA2	**miR124**	**-22.846**	**-24.995**	**-20.697**	**< 0.001**

B



C

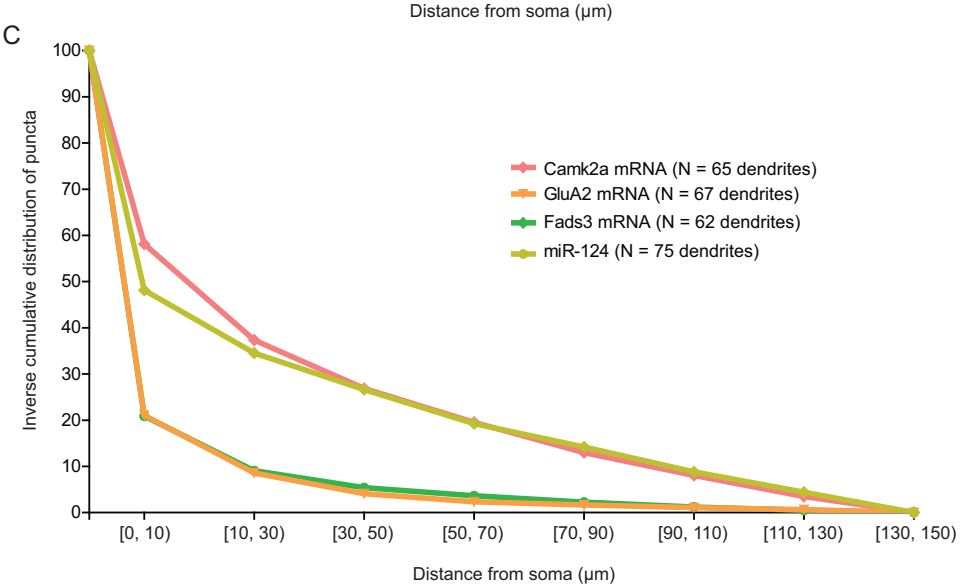


Figure S3.

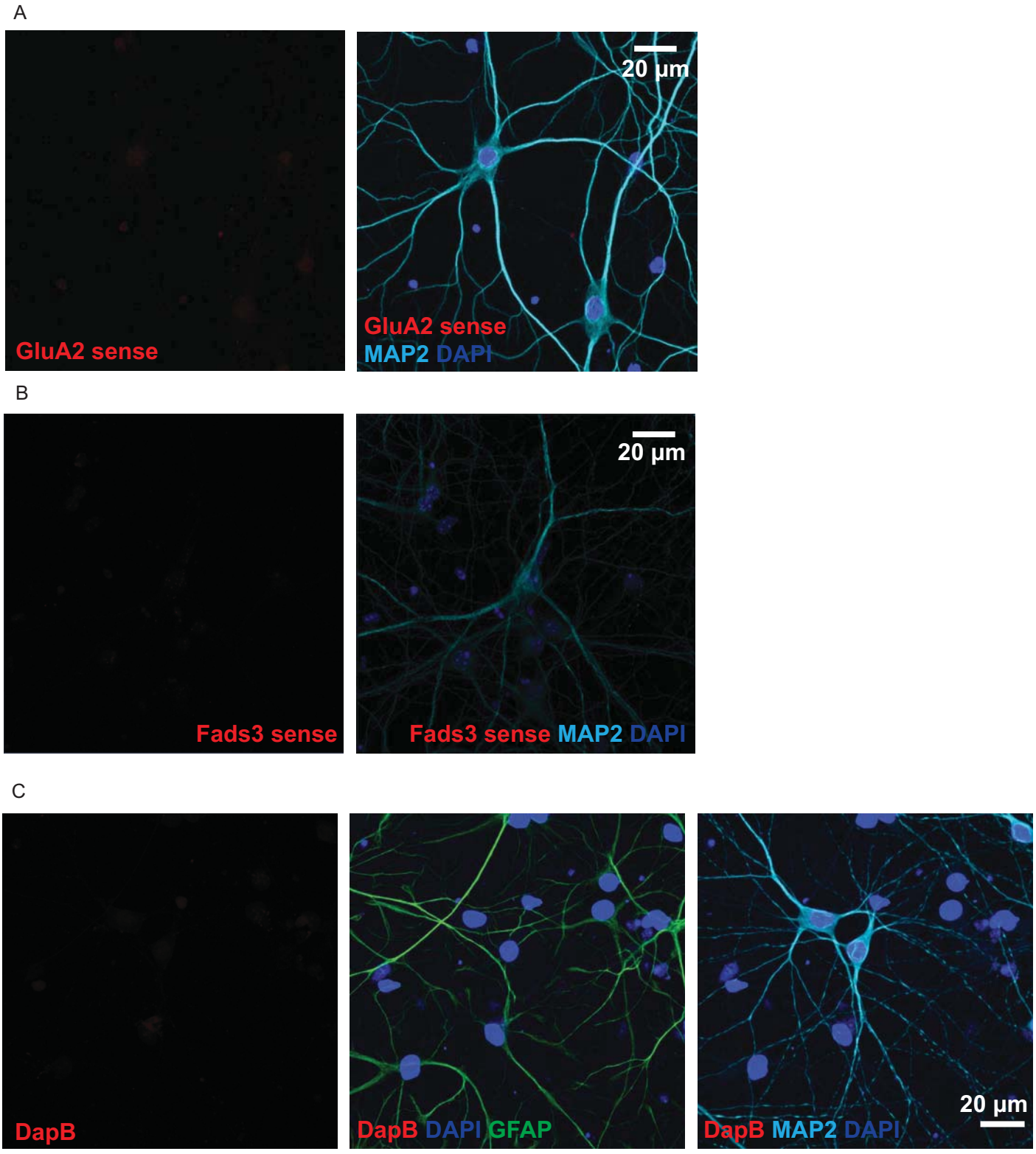

A
GluA2 sense
20 µm
GluA2 sense
MAP2 DAPI
B
Fads3 sense
20 µm
Fads3 sense MAP2 DAPI
C
DapB
DapB DAPI GFAP
DapB MAP2 DAPI
20 µm

Figure S4.

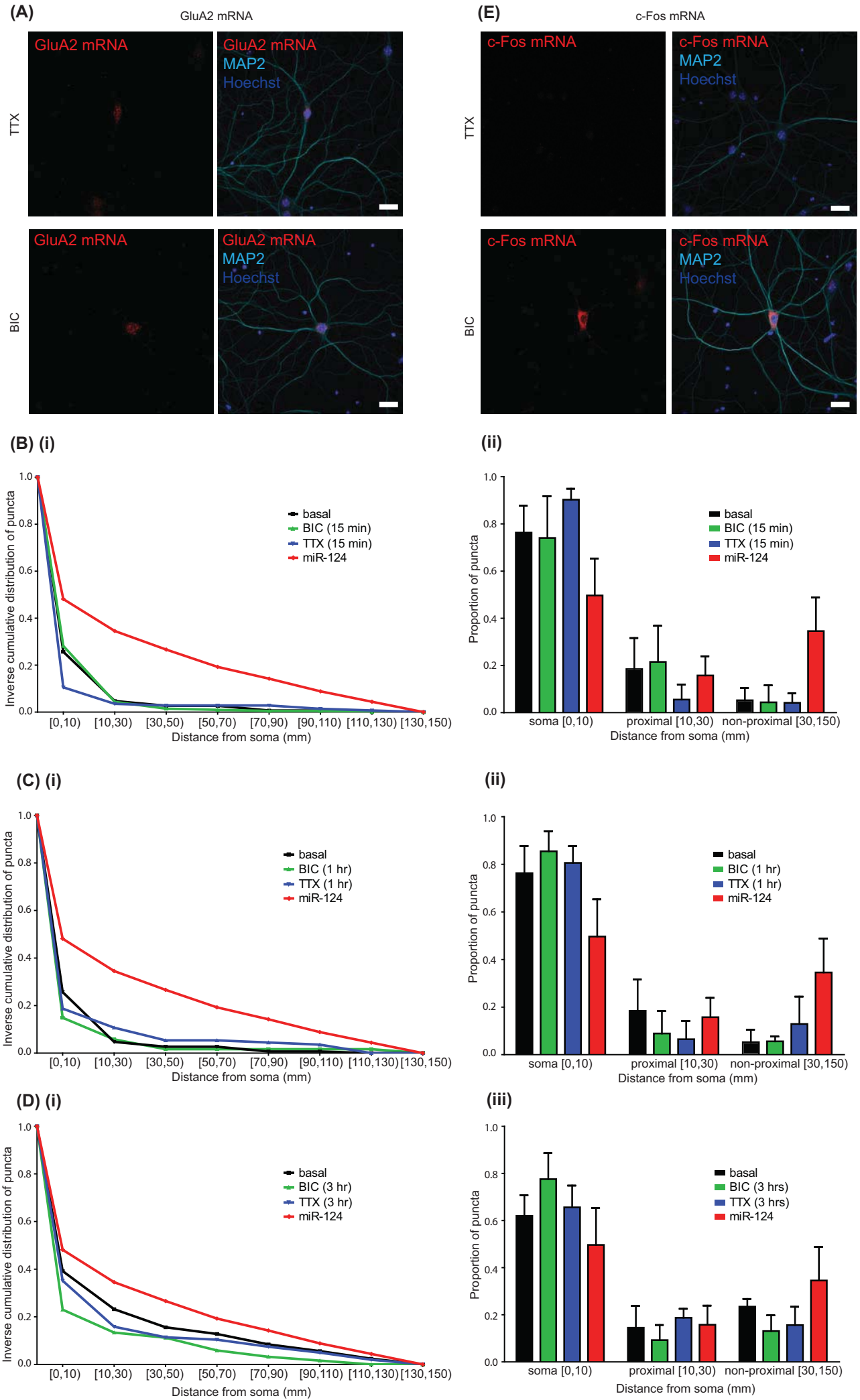

(A)
GluA2 mRNA
GluA2 mRNA
MAP2
Hoechst
TTX
BIC
(E)
c-Fos mRNA
c-Fos mRNA
MAP2
Hoechst
(B) (i)
Inverse cumulative distribution of puncta
basal
BIC (15 min)
TTX (15 min)
miR-124
[0,10) [10,30) [30,50) [50,70) [70,90) [90,110) [110,130) [130,150)
Distance from soma (mm)
(ii)
Proportion of puncta
soma [0,10)
proximal [10,30)
non-proximal [30,150)
(C) (i)
BIC (1 hr)
TTX (1 hr)
(ii)
(D) (i)
BIC (3 hr)
TTX (3 hr)
(iii)
BIC (3 hrs)
TTX (3 hrs)

Figure S5.

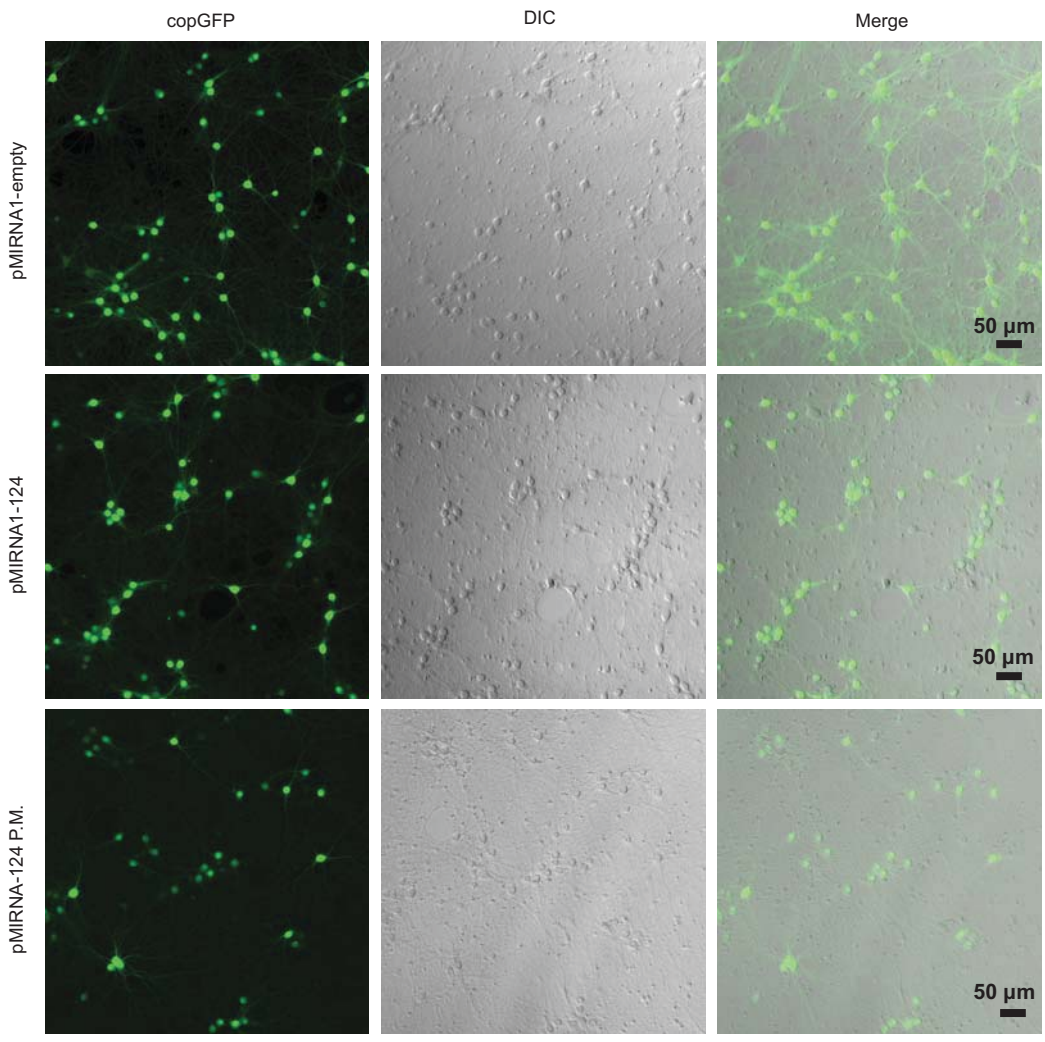
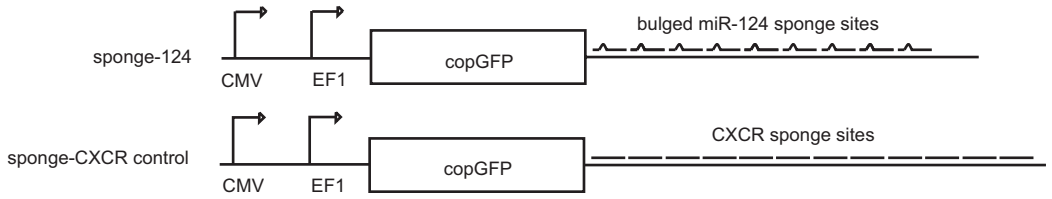
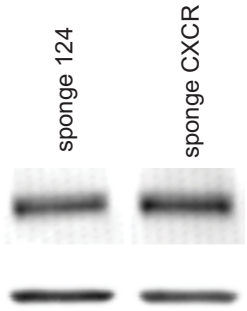

copGFP
DIC
Merge
pMIRNA1-empty
pMIRNA1-124
pMIRNA-124 P.M.
50 µm
50 µm
50 µm

Figure S6.

A



B



0.98 ± 0.28

C

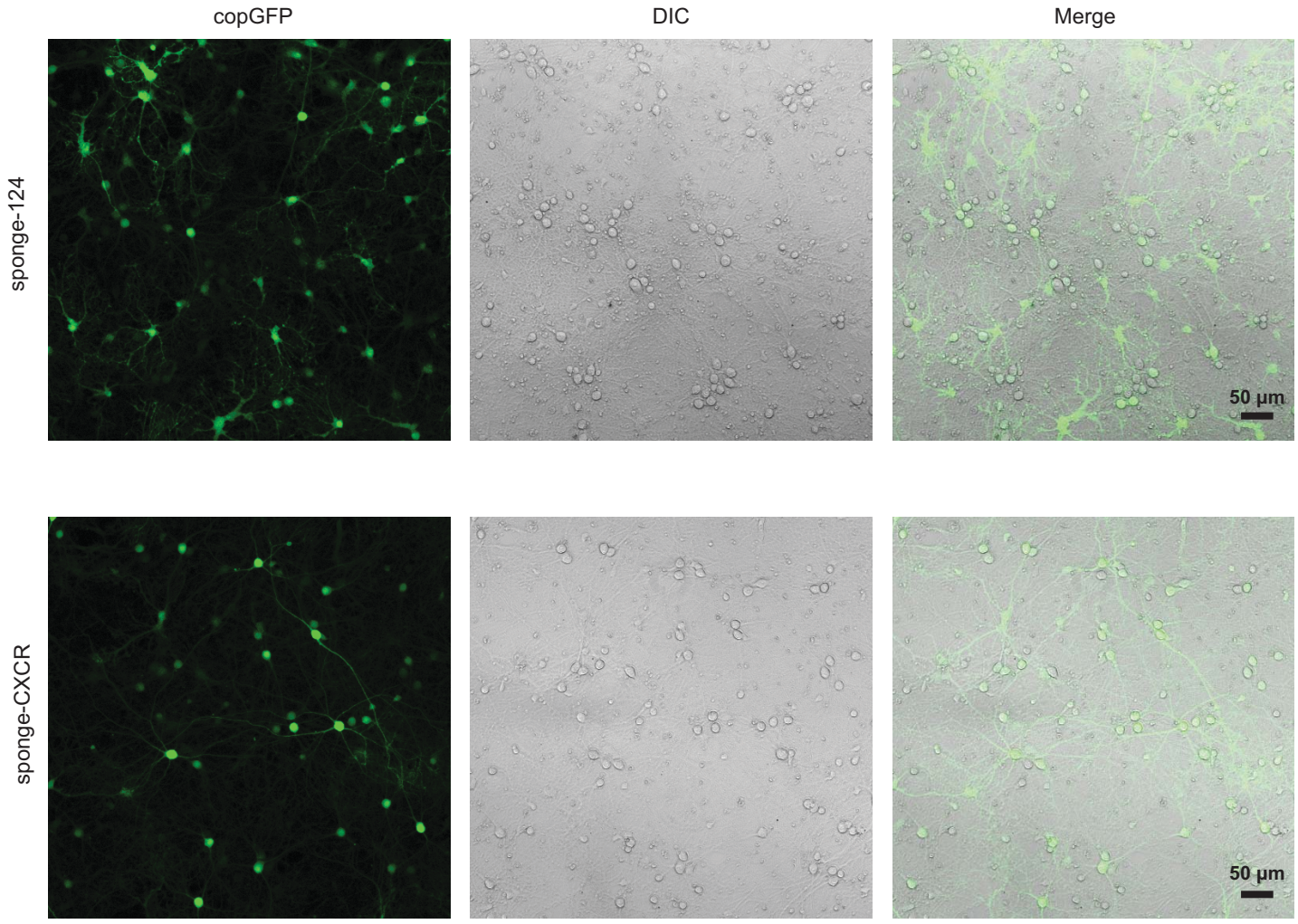


Figure S7.

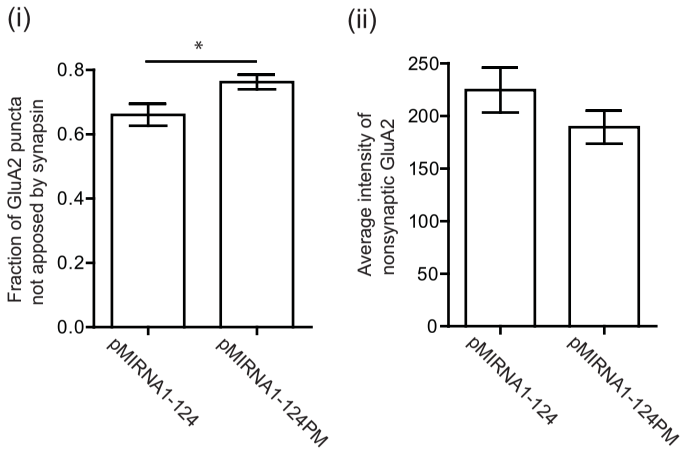

(i)
*
Fraction of GluA2 puncta not apposed by synapsin
0.8
0.6
0.4
0.2
0.0
pMIRNA1-124
pMIRNA1-124PM
(ii)
Average intensity of nonsynaptic GluA2
250
200
150
100
50
0
pMIRNA1-124
pMIRNA1-124PM

Figure S8.

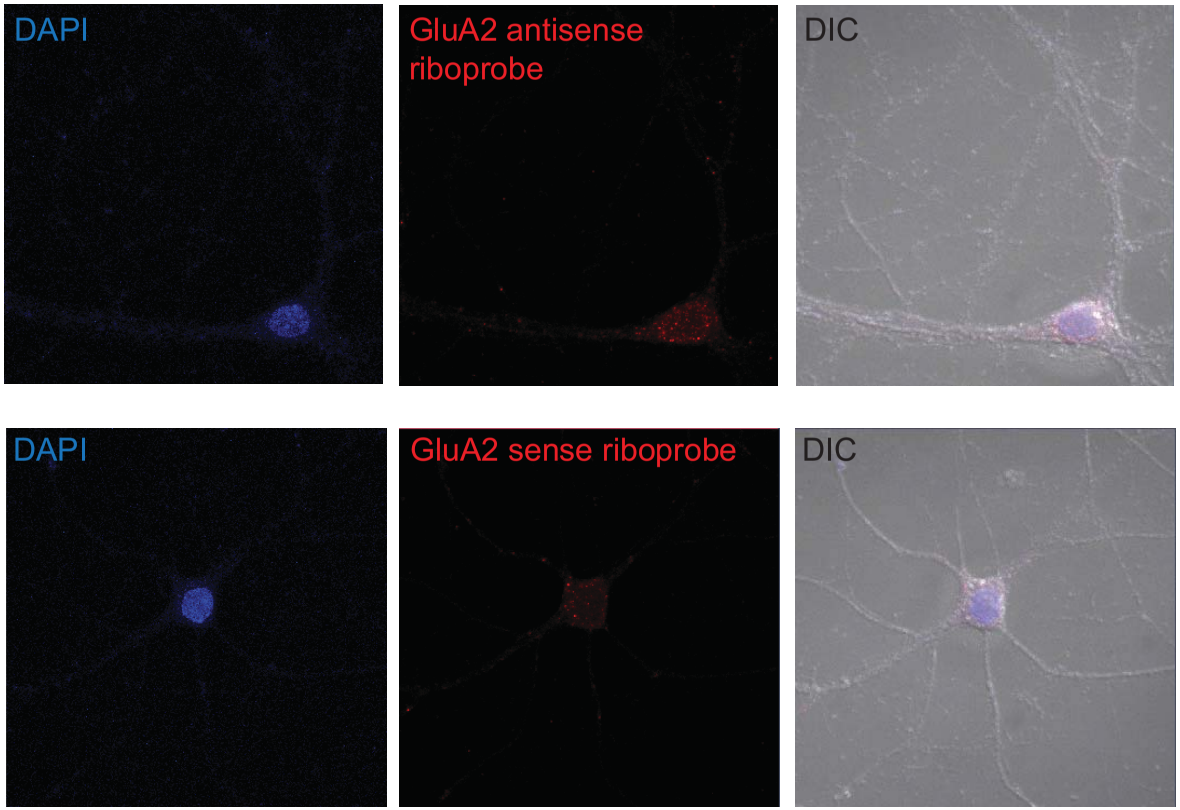

DAPI
GluA2 antisense riboprobe
DIC
DAPI
GluA2 sense riboprobe
DIC

Figure S9.

(A)

		miR-124	
Experiment	Condition	Ct	Fold change
1	TTX (12 hr)	27.05	1.08
	basal	27.15	1.00
	BIC + FSK (1 hr)	26.76	1.31
2	TTX (12 hr)	27.24	0.87
	basal	27.04	1.00
	BIC + FSK (1 hr)	27.04	1.00
3	TTX (12 hr)	25.81	1.00
	basal	25.81	1.00
	BIC + FSK (1 hr)	25.74	1.05

(B)