Supplemental Data

Neurofibromin Regulation of ERK Signaling Modulates GABA Release and Learning

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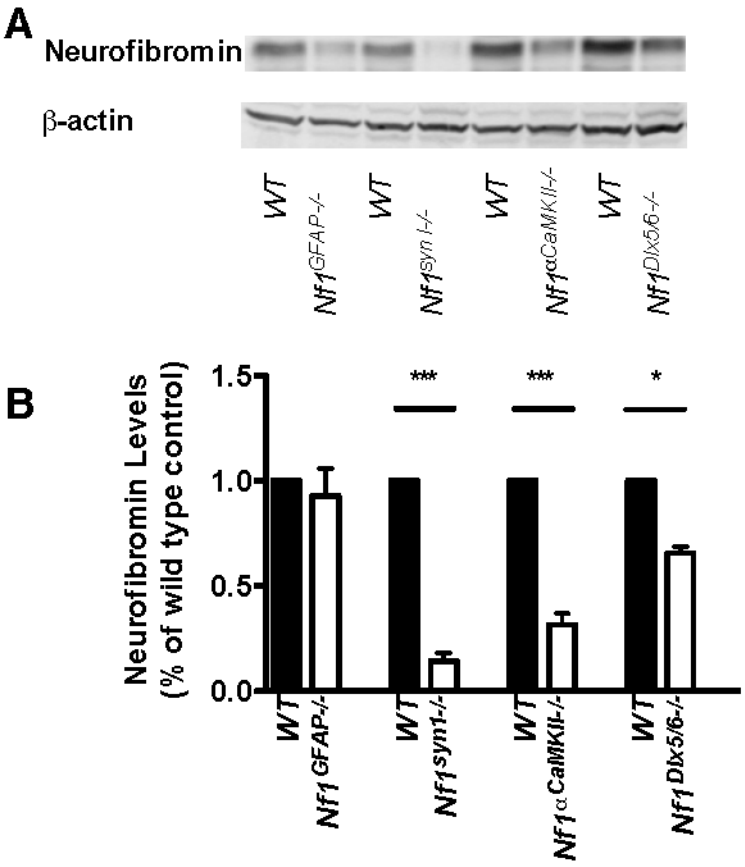

Figure S1. Levels of neurofibromin in hippocampal CA1 area of Cre-mediated *Nf1* conditional mouse mutants

A. Representative Western blot analyses (anti-NF1GRD antibody) of Hippocampal CA1 neurofibromin expression in homozygous conditional cre-mediated *Nf1* mutants and their littermate WT mice. β-actin signal was used as a loading control. B. Quantification of hippocampal CA1 neurofibromin expression level in $Nf1^{GFAP-/-}$, $Nf1^{syn\ I-/-}$, $Nf1^{\alpha CaMKII-/-}$, and $Nf1^{Dlx5/6-/-}$, mice and their WT controls (3-5 mice in each group). A Student's t test with a critical value of $p<0.05$ was used for all statistical analyses. Values are mean ± SEM, * $P<0.05$, ***$P<0.001$.

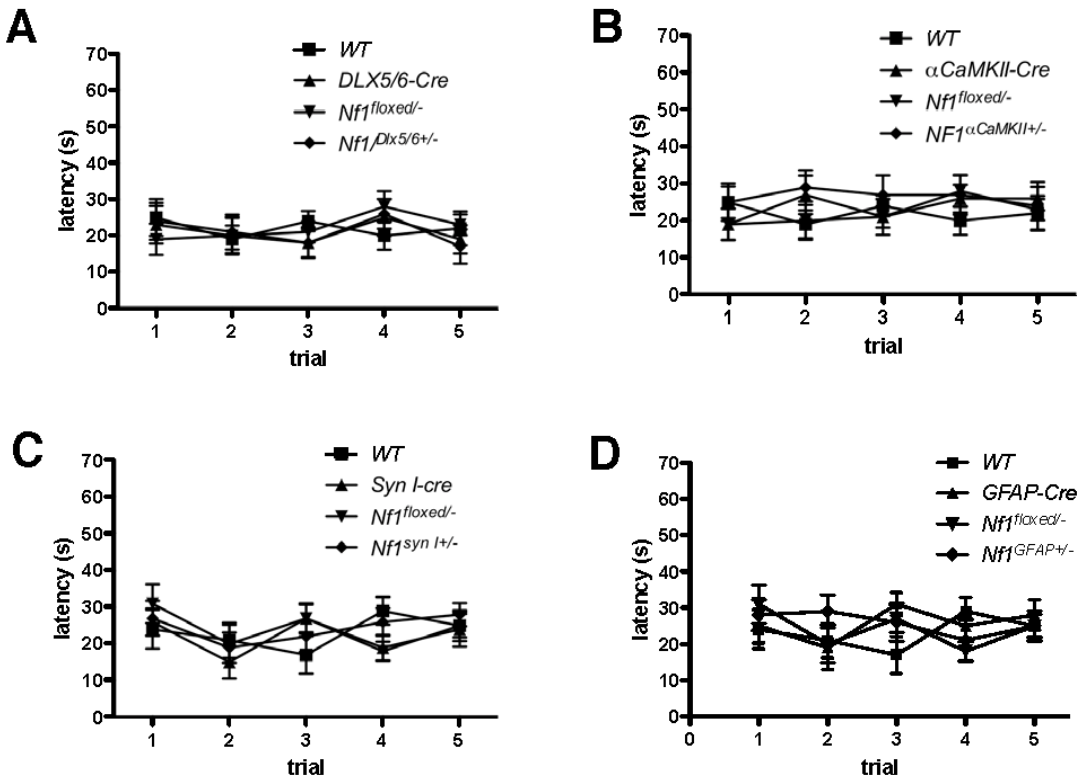

Figure S2. Visible platform test escape latency values for Cre-mediated *Nf1* conditional mutants

The average escape latency during visible platform test (two trials averaged for each animal after hidden platform training) is plotted for $Nf1^{cre+/-}$ mice and WT littermates. A. $Nf1^{Dlx5/6+/-}$ (n=8) mice and littermates controls; B. $Nf1^{\alpha CaMKII+/-}$ (n=8) and littermates controls; C. $Nf1^{syn\ I+/-}$ (n=13)mice and littermates controls; D. $Nf1^{GFAP+/-}$ (n=12) mice and littermates controls. Error bars indicate SEM.

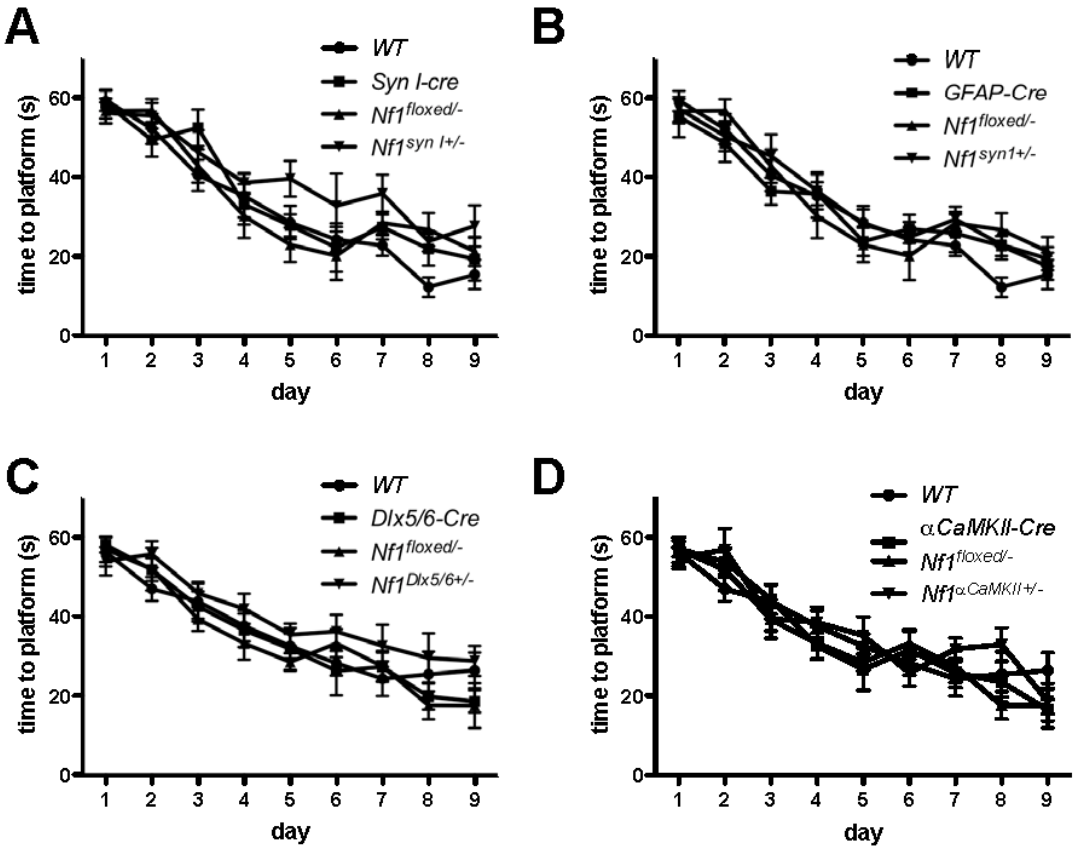

Figure S3. Water maze latency values for Cre-mediated *Nf1* conditional mutants
$Nf1^{cre+/-}$ mice and WT littermates were trained with 2 trials per day in the water maze. The average latency to reach the hidden platform during training is plotted. A. $Nf1^{syn\ I+/-}$ (n=13) mice and littermates controls; B. $Nf1^{GFAP+/-}$ (n=12) and littermates controls; C. $Nf1^{Dlx\ 5/6+/-}$ (n=8) mice and littermates controls; D. $Nf1^{\alpha CaMKII+/-}$ (n=8) mice and littermates controls. Error bars indicate SEM.

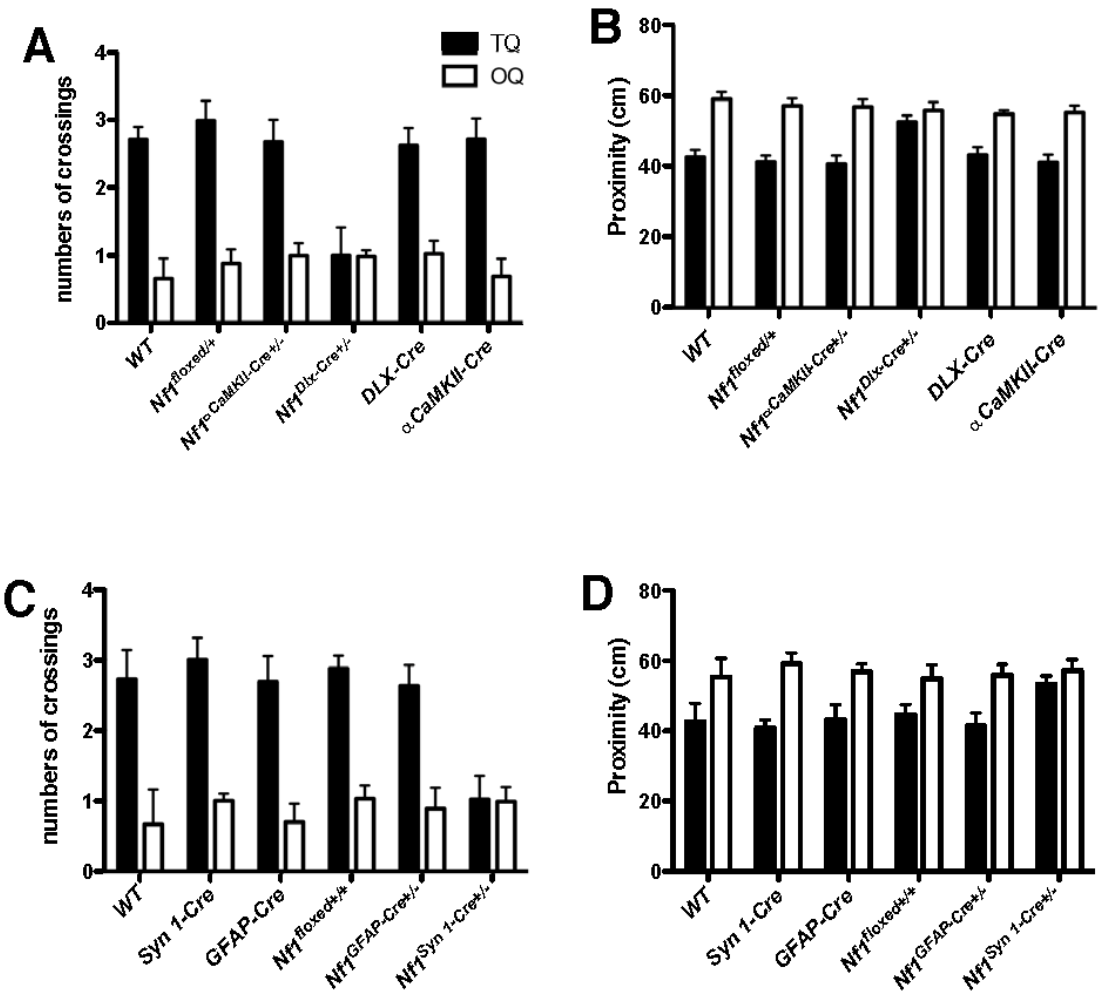

Figure S4. Cre-deletions of neurofibromin gene in inhibitory neurons cause learning deficits.

A. Number of times the mice crossed the exact position where the platform was during the training (solid bar), compared with the number of crossings in the opposite position in the pool (open bar) for $Nf1^{Dlx5/6+/-}$, $Nf1^{\alpha CaMKII+/-}$ and their littermates controls. B. Average proximity to the exact position where the platform was during training (solid bar), compared with proximity to the opposite position in the pool (open bar) for $Nf1^{Dlx5/6+/-}$, $Nf1^{\alpha CaMKII+/-}$ and their littermates controls. C. Number of times the mice crossed the exact position where the platform was during the training (solid bar), compared with the number of crossings in the opposite position in the pool (open bar) for $Nf1^{syn\ I+/-}$, $Nf1^{GFAP+/-}$ and their littermates controls. D. Average proximity to the exact position where the platform was during training (solid bar), compared with proximity to the opposite position in the pool (open bar) for $Nf1^{syn\ I+/-}$, $Nf1^{GFAP+/-}$ and their littermates controls. Values are mean ± SEM.

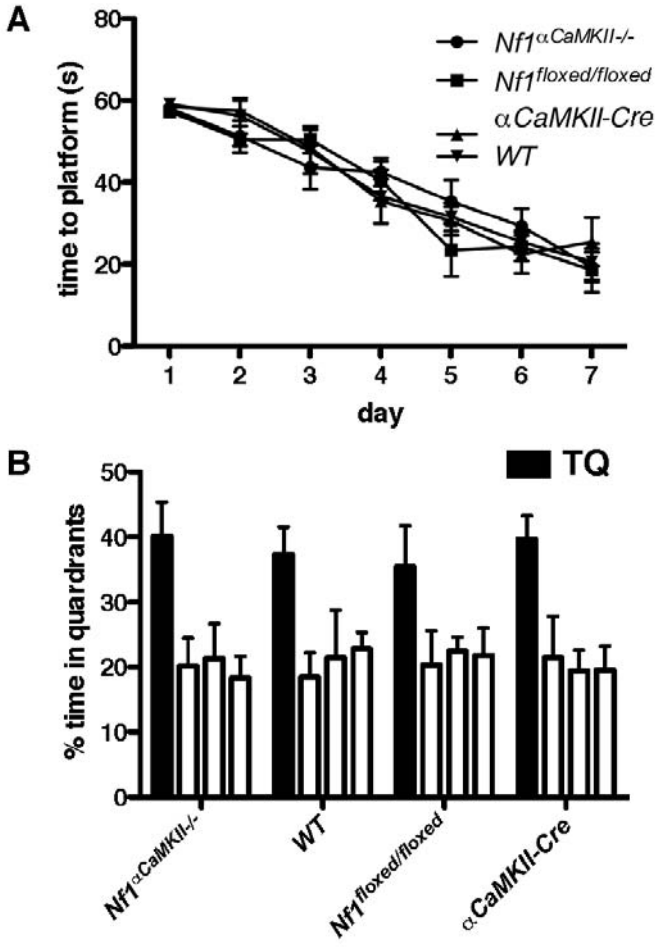

Figure S5. Spatial learning of the *Nf1*$^{\alpha CaMKII-/-}$ mice in the water maze

A. *Nf1*$^{\alpha CaMKII-/-}$ mice (n=12) and littermate controls were trained for 2 trials per day in the water maze. The average latency to reach the hidden platform is plotted. There is no difference in latencies between mutants and controls during training; B. Results of a probe trial given after 7 days of training. The percentage of time animals spent searching in each of the training quadrants is shown. Both *Nf1*$^{\alpha CaMKII-/-}$ mutants ($F_{(3,44)}$=17.328, P<0.05) and controls searched selectively and spent significantly more time searching in the training quadrant than in any of the other quadrants. However, there was no significant difference between the two groups ($F_{(3,40)}$=0.45, P>0.05). Values are mean ± SEM.

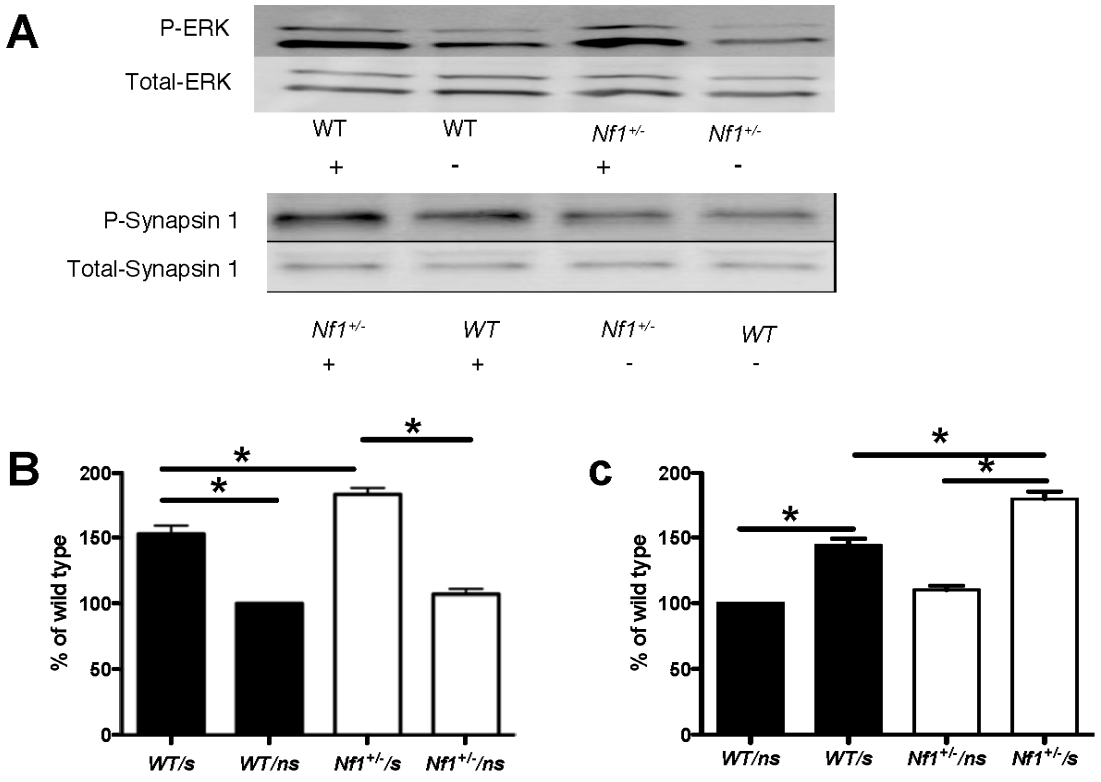

Figure S6. Increased ERK-dependent synapsin I phosphorylation in *Nf1*$^{+/-}$ mice. Contextual conditioning increases the phosphorylation of ERK and synapsin I (sites 4/5). A. Representative Western blots indicating protein bands visualized with antibodies to dually phosphorylated ERK1/2, total ERK1/2, synapsin I at sites 4/5, and total ERK1/2. The total ERK1/2 and total synapsin I levels indicate equal protein loading. "+" symbols denote contextual conditioning (shocks were delivered during the contextual exposure)." –" symbols denote that no shock was delivered. B and C: Quantification of relative phosphorylation of ERK1/2, and synapsin I at sites 4/5; For each experiment, both phosphorylated and total MAPK levels were normalized to those observed in the control group of wild-type mice. we used 3-6 mice in each group. Values are mean ± SEM.

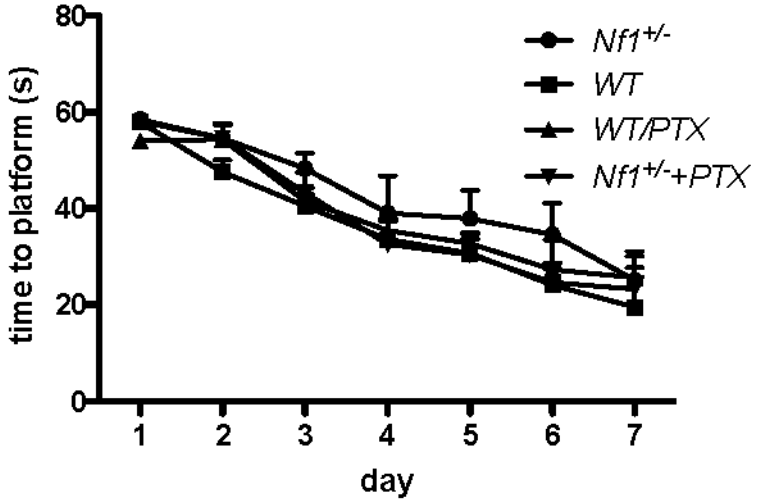

Figure S7. Water maze latency values for *Nf1*+/- mice treated with picrotoxin
Nf1$^{+/-}$ mice and WT littermates were trained with 2 trials per day in the water maze. The average latency to reach the hidden platform during training is plotted. Error bars indicate SEM.

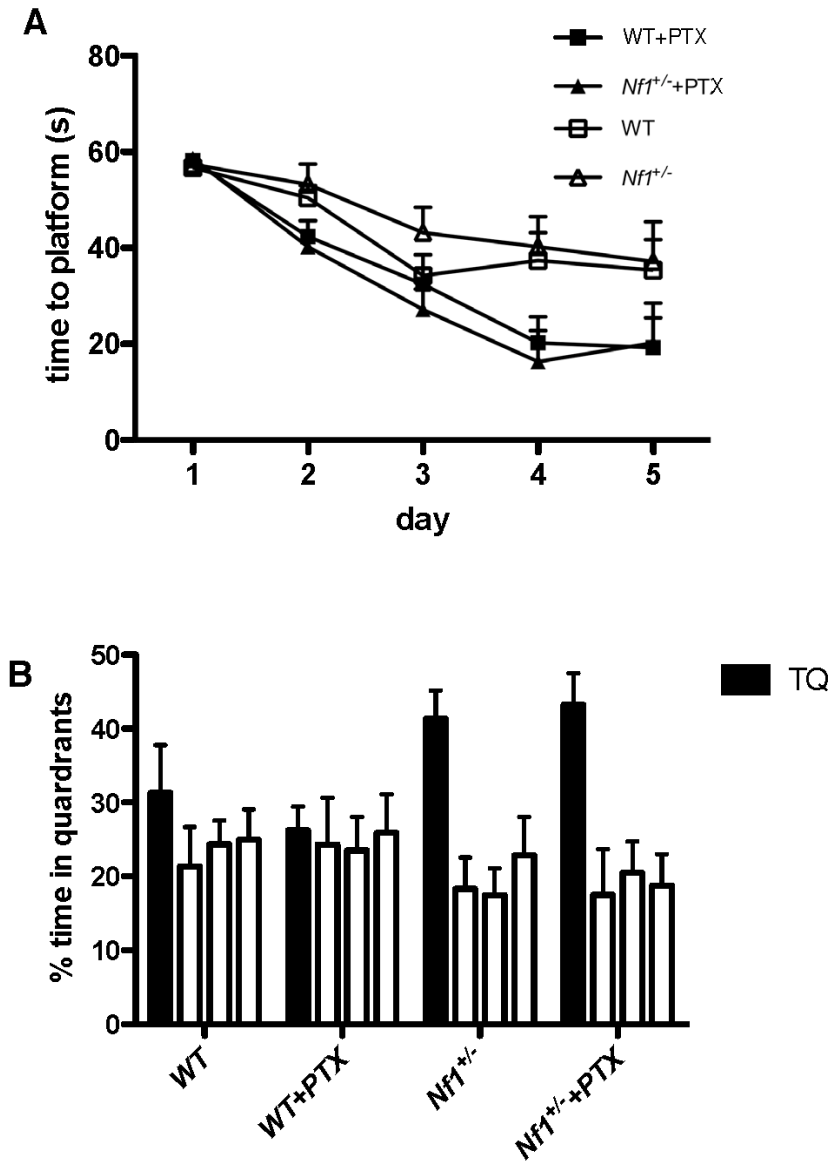

Figure S8: Spatial learning in the water maze of *Nf1*$^{+/-}$ mice treated with 0.03mg/Kg of picrotoxin

Nf1$^{+/-}$ mice and WT littermates were treated with either saline or picrotoxin (0.03mg/Kg) trained with 2 trials per day in the water maze. The average latency to reach the hidden platform (A) and % time in quadrants (B) is plotted. The dose in these experiments (0.03mg/Kg) is higher that the dose used in Fig.6 (0.01mg/Kg); this higher dose (0.03mg/Kg) enhances the spatial learning of both mutants and controls. Values are mean ± SEM.

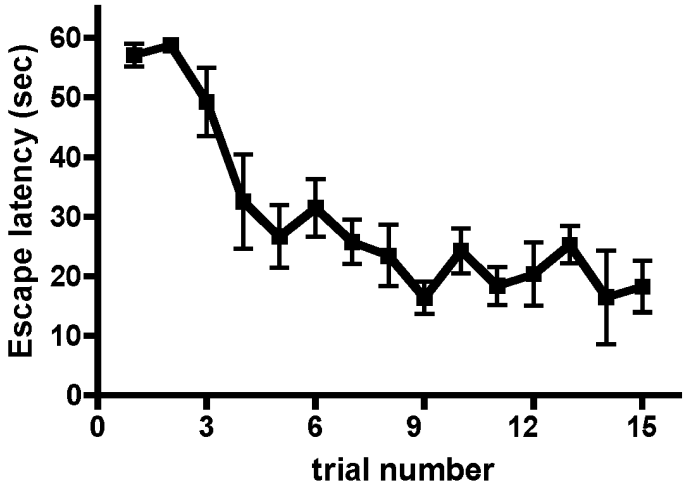

Figure S9: Water maze training results for the mice used in Fig. 7
Mice were trained in the Morris water maze with 15 trails in one day. The latency to get to the platform was significantly decreased with training. Values are mean ± SEM.

Table S1. Floating, hug time average swimming speed of the Nf1 mutant lines and their controls during the day 7 probe trial

Mouse Lines	No. of Mice	No. of floaters	Hug Time (%)	Swim speed(cm/s)
Nf1$^{syn1+/-}$				
WT*	16	0	15.4±3.8	19.2±1.6
Syn1-Cre	14	0	18.4±4.9	20.4±4.8
*Nf1-floxed***	15	1	18.2±5.2	18.8±5.1
Nf1$^{syn1+/-}$	13	1	16.5±3.6	17.4±3.1
Nf1$^{gfap+/-}$				
WT*	16	0	15.4±3.8	19.2±1.6
GFAP-Cre	13	0	16.4±4.9	15.4±4.1
*Nf1-floxed***	15	1	18.2±5.2	18.8±5.1
Nf1$^{gfap+/-}$	12	0	17.8±6.3	16.8±4.7
Nf1$^{aCaMKII+/-}$				
WT***	8	0	15.4±4.8	18.6±3.7
□*CaMKII-Cre*	8	1	14.3±3.9	17.5±3.7
*Nf1-floxed****	7	0	18.5±3.8	19.6±5.4
Nf1$^{aCaMKII+/-}$	8	0	16.2±4.3	19.7±3.9
Nf1$^{Dlx+/-}$				
WT ***	8	0	15.4±4.8	18.6±3.7
Dlx5/6-Cre	7	0	17.2±4.8	16.4±3.7
*Nf1-floxed****	7	0	18.5±3.8	19.6±5.4
Nf1$^{Dlx5/6+/-}$	8	0	19.1±3.4	15.4±4.3

*: same group of mice
**: same group of mice
***: same group of mice
****: same group of mice

Table S2. Statistic results of the performance of each mutant and controls in the Morris water maze

Mouse Lines	F-Value	P-Value
Nf1$^{syn1+/-}$		
WT*	$F_{(3,60)}$ =11.677	<0.001
Syn1-Cre	$F_{(3,52)}$ =9.481	0.001
*Nf1-floxed***	$F_{(3,51)}$ =17.671	<0.001
Nf1$^{syn1+/-}$	$F_{(3,48)}$ =0.045	0.987
Nf1$^{gfap+/-}$		
WT*	$F_{(3,60)}$ =11.677	<0.001
GFAP-Cre	$F_{(3,48)}$ =11.040	0.001
*Nf1-floxed***	$F_{(3,51)}$ =17.671	<0.001
Nf1$^{gfap+/-}$	$F_{(3,41)}$ =7.873	<0.001
Nf1$^{aCaMKII+/-}$		
WT***	$F_{(3,28)}$ =10.127	<0.001
□*CaMKII-Cre*	$F_{(3,28)}$ =4.208	<0.05
*Nf1-floxed****	$F_{(3,24)}$ =10.806	<0.001
Nf1$^{aCaMKII+/-}$	$F_{(3,28)}$ =12.102	<0.001
Nf1$^{Dlx+/-}$		
WT***	$F_{(3,28)}$ =10.127	<0.001
Dlx-Cre	$F_{(3,24)}$ =4.866	<0.001
*Nf1floxed****	$F_{(3,24)}$ =10.806	<0.001
Nf1$^{Dlx+/-}$	$F_{(3,28)}$ =0.458	0.7141

*: same group of mice
**: same group of mice
***: same group of mice
****: same group of mice

Table S3. Water maze statistical results for conditional mutants and their controls in target quadrant

Mouse lines	F-Value	P-Value
Nf1$^{syn1+/-}$	$F_{(3,54)}$ =9.755	<0.001
Nf1$^{gfap+/}$	$F_{(3,52)}$ =0.626	0.6014
Nf1$^{aCaMKII+/-}$	$F_{(3,27)}$=0.759	0.5268
Nf1$^{Dlx+/-}$	$F_{(3,26)}$=5.437	<0.05

Table S4. Properties of sIPSCs and mIPSCs in CA1 Pyramidal Neurons of *Nf1*$^{+/-}$ mice

	Amplitude (pA)	Frequency (Hz)	RT 10%-90% (ms)	τw (ms)	No. Cells
sIPSCs					
WT	69.54±2.54	18.25±2.39	0.76±0.07	7.15±0.72	12
Nf1$^{+/-}$	6.97±0.83	65.43±5.35	20.78±2.10	0.80±0.09	14
mIPSCs					
WT	48.38±4.38	13.89±0.69	0.67±0.04	6.88± 0.40	30
WT+High K^+ ACSF	47.29±3.46	21.98±0.95	0.71±0.06	6.47± 0.96	30
Nf1$^{+/-}$	48.31±5.61	16.25±0.65	0.69±0.05	6.48± 0.52	29
Nf1$^{+/-}$+ High K^+ ACSF	46.72.±6.17	43.21±1.11	0.67±0.06	6.72±1.57	29

Table S5. Floating, hug time average swimming speed of the *Nf1*$^{+/-}$ and WT mice during the day 7 probe trial

Mouse Group	No. of Mice	No. of floaters	Hug Time (%)	Swim speed(cm/s)
WT	19	0	15.1±4.7	18.0±3.9
WT+picrotoxin	19	1	16.1±5.9	19.3±5.2
Nf1$^{+/-}$	19	0	17.1±5.1	17.9±4.8
Nf1$^{+/-}$+picrotoxin	18	1	16.9±4.7	18.8±5.1