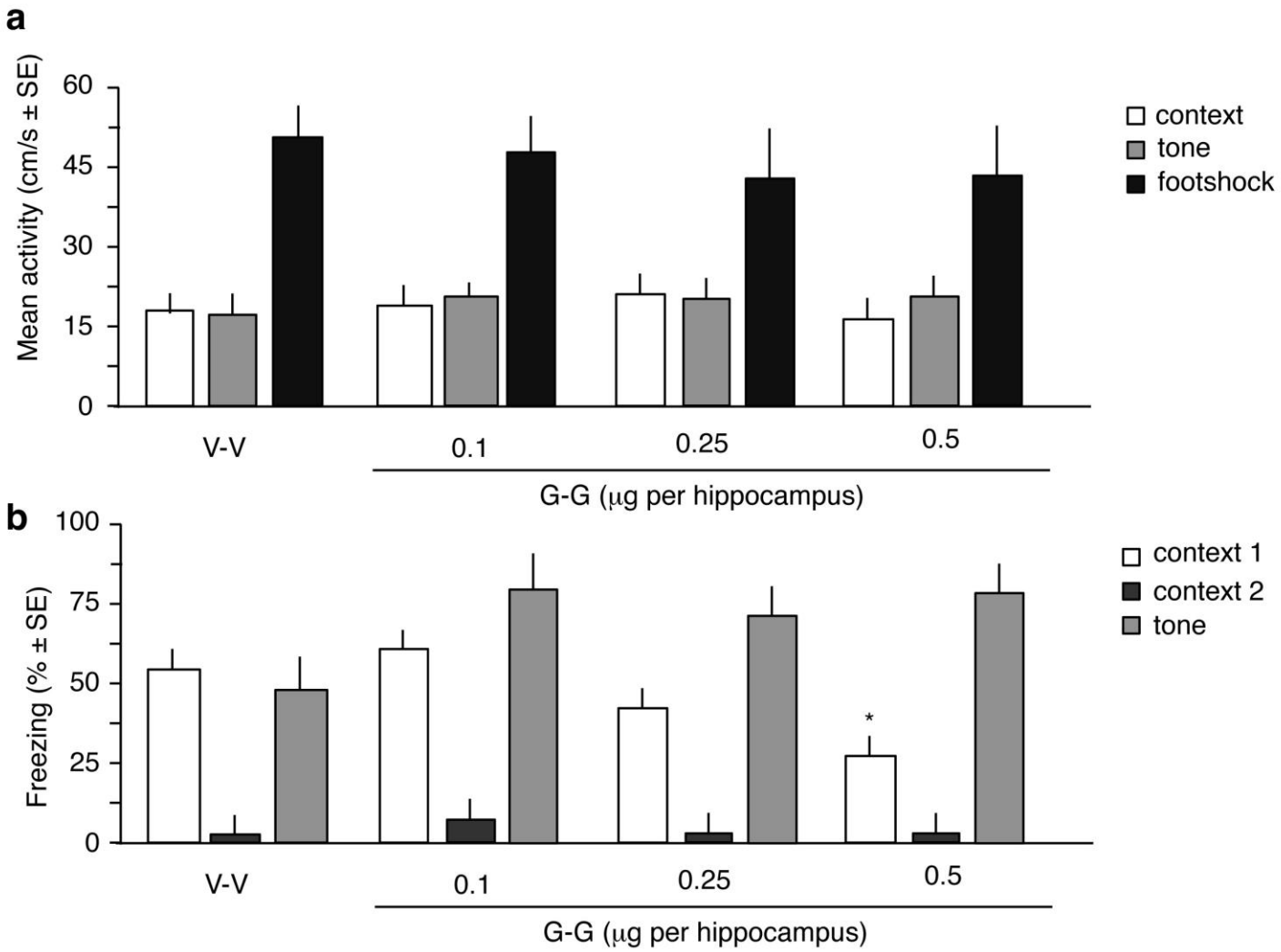
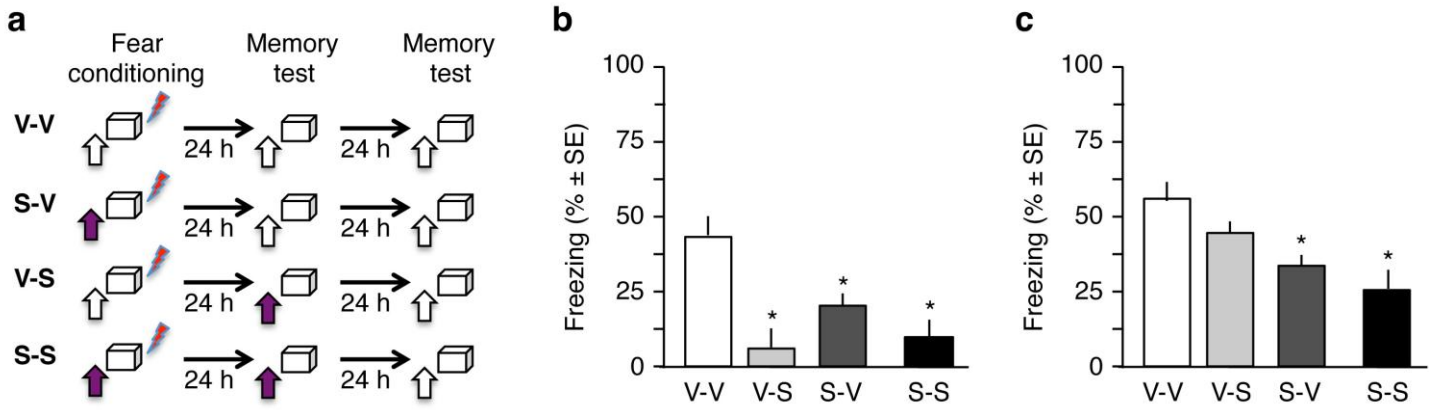


Supplementary Figure 1

Gaboxadol does not affect tone-dependent fear conditioning after i.h. injection.

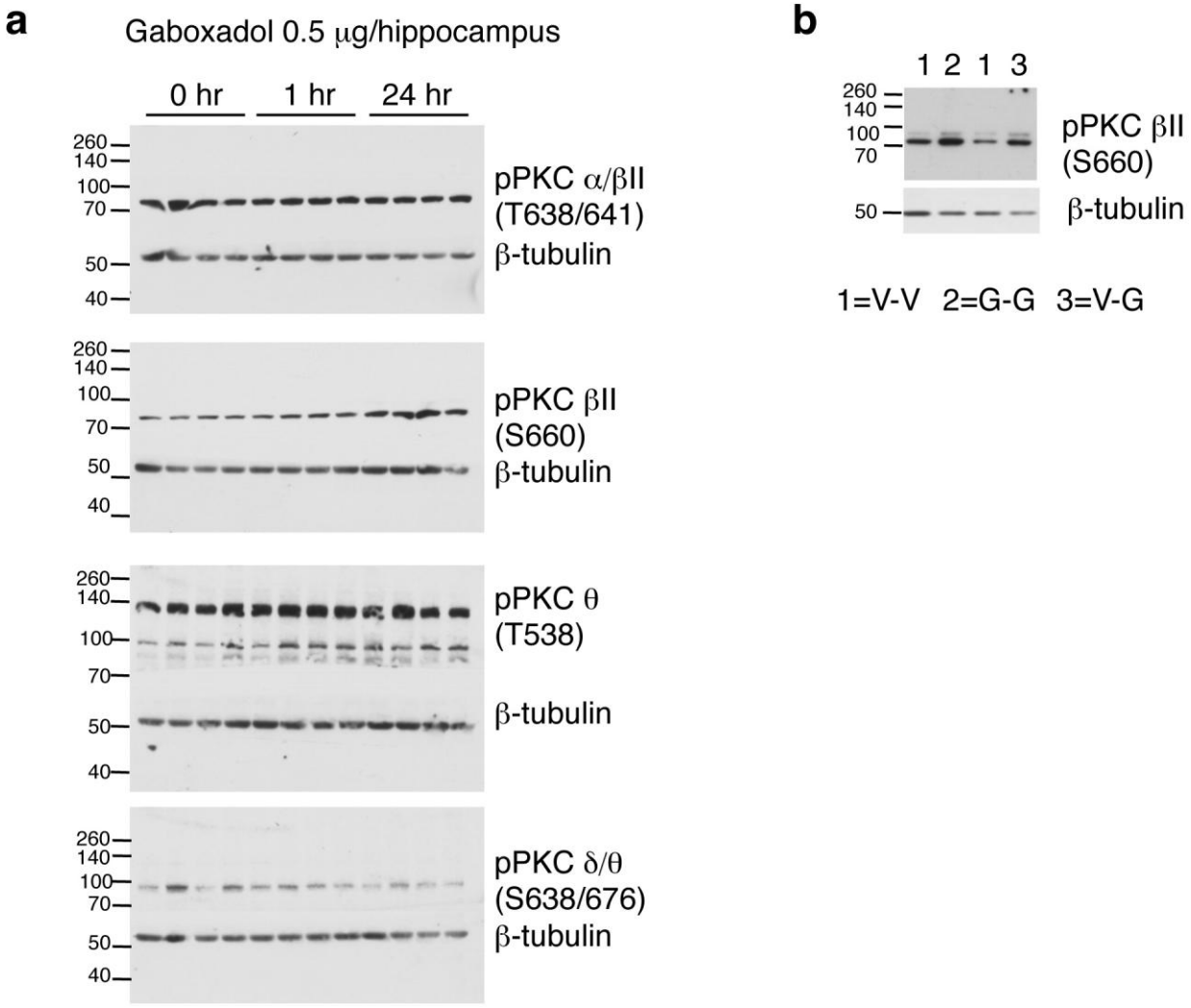
(**a**) Gaboxadol (0.1-0.5 μg/hippocampus) did not affect locomotor activity to context or tone at training or activity burst to the footshock (n = 8 mice per group; context 1: $F_{3,22} = 0.814$, $P = 0.499$; tone: $F_{3,22} = 0.431$, P = 0.733; shock: $F_{3,22} = 1.109$, $P = 0.366$). (**b**) In contrast to reduced freezing in context 1, freezing to tone and contextual generalization were unchanged in response to gaboxadol (n = 8 mice per group; context 1: $F_{3,22} = 3.742$, $P < 0.05$; context 2: $F_{3,22} = 0.947$, $P = 0.434$; tone: $F_{3,22} = 0.426$, $P = 0.737$). *$P < 0.01$ vs vehicle.



Supplementary Figure 2

Scopolamine does not induce state-dependent contextual fear conditioning.

(**a**) Treatment schedule with scopolamine (25 µg/hippocampus). (**b**) Scopolamine significantly impaired freezing in all groups including the S-S group, indicating lack of state-dependent contextual fear at doses that impair fear conditioning and memory retrieval ($F_{3,28}$ = 8.810, P < 0.001). (**c**) Freezing in the V-S group recovered when mice were tested off drug, whereas the S-V and S-S groups maintained the freezing deficits $F_{3,28}$ = 3.444, P < 0.05. *P < 0.01 vs V-V group (n = 8 mice/group).



Supplementary Figure 3

Full length images of immunoblots showing increased phosphorylation of PKC βII.

(**a**) Phosphorylation of PKC isoforms 1 and 24 hrs after fear conditioning. (**b**) Phosphorylation of PKC βII immeadiately after testing of V-G nd G-G mice.

a

miRNA	Fold change*	GABAA receptor targets (www.targetscan.org, microRNA.org)
miR-33	2.21	GABRA4, GABRB2, GABRG1, GABRP, GABRG2, GABRB3
miR-193	2.05	GABRE
miR-136	2.03	GABRG2, GABRB2, GABRG1, GABRB3
miR-153	2.00	GABRG1, GABRA4
miR-381	1.85	GABRE, GABRG1, GABRA5, GABRA4, GABRA3
miR-376a	1.80	GABRB2
miR-127*	1.79	n.a.**
miR-29b	1.77	GABRE, GABRR1
miR-136*	1.67	n.a.
miR-339-5p	1.64	no known GABAR targets
miR-24-2*	1.61	n.a.
miR-29c	1.60	GABRE, GABRR1
miR-144	1.60	GABRB2, GABRA6, GABRG1, GABRA4
miR-124	1.58	GABRA6
miR-551b	1.57	no known GABAR targets
miR-219	1.56	GABRA4, GABRB2
miR-29a*	1.53	n.a.
miR-142-3p	1.51	GABRA3, GABRB2, GABRB3
miR-494	0.41	GABRA5, GABRA3, GABRAQ, GABRG3, GABRP, GABRB3, GABRA4

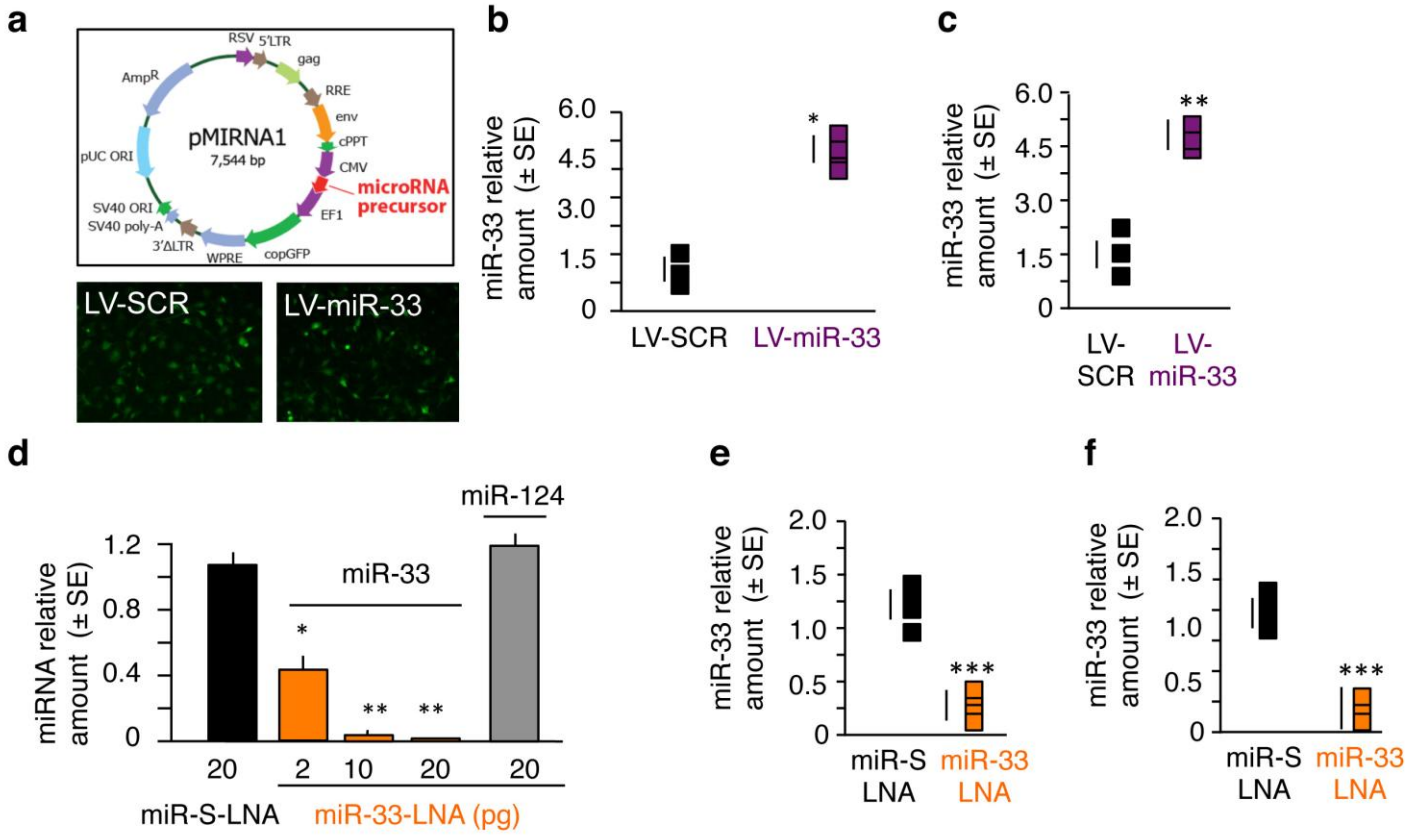
*fear conditioned vs naïve mice; ** n.a., data not available

b

Supplementary Figure 4

Differential expression of microRNAs that target GABA-related proteins during fear conditioning.

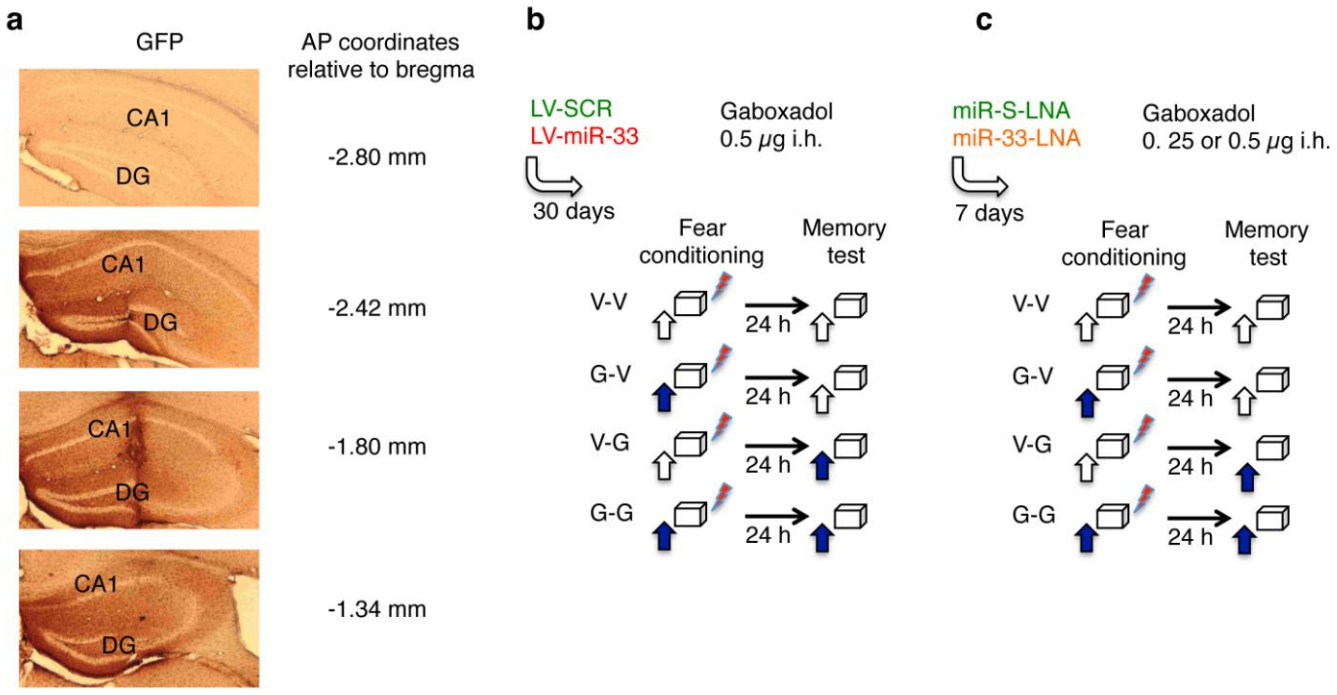
(**a**) Microarrays of microRNAs differentially expressed in hippocampi of mice after fear conditioning (F) that show > 50% change when compared to control hippocampi from naïve (N) mice. Of nineteen identified microRNAs, fourteen have predicted targets within $GABA_A$ receptors, and five of them (marked red) have four or more predicted GABAR targets. (**b**) Conserved miR-33 targets in GABA-related proteins.



Supplementary Figure 5

Validation of the miR-33 manipulation.

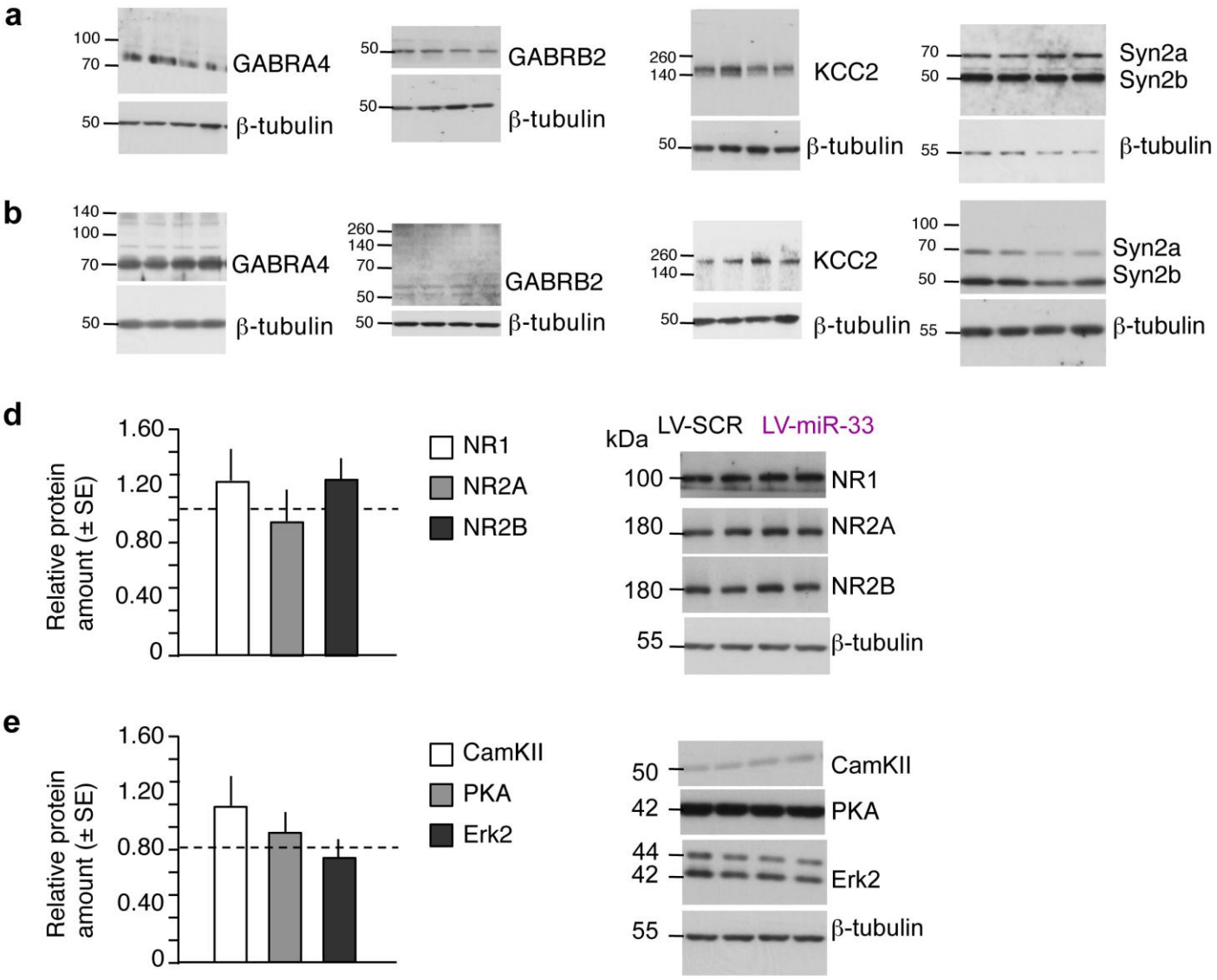
(**a**) The plasmid construct carrying miR-33 indicates lack of viral toxicity as revealed by viable and healthy neuroblastoma cells infected with LV-SCR or LV-miR-33. (**b**) qPCR analysis of relative miR-33 level in cells infected with LV-miR-33 vs LV-SCR (t_6 = -3.508, $P < 0.05$, t-test). (**c**) In vivo validation of the miR-33 overexpression in the mouse hippocampus obtained from mice tested as described in Fig. 3b (n = 5 hippocampi/group; t_9=10.297, $P < 0.01$). (**d**) Dose-dependent inhibition of miR-33 after i.h. injection of miR-33 LNA when compared to miR-S-LNA ($F_{4,14}$ = 48.368, $P < 0.001$, one-way ANOVA). miR-124 levels were determined as a specificity control. (**e**) and (**f**) In vivo validation of the miR-33 down-regulation in the mouse hippocampus obtained from mice tested as described in Fig. 3c,d (n = 4 hippocampi/group; t_7=8.715, $P < 0.01$ and n = 4 hippocampi/group; t_7=9.12, $P < 0.001$). *$P < 0.05$, **$P < 0.01$, *** $P < 0.001$ vs corresponding controls.



Supplementary Figure 6

Virus spread and schematic representation of the treatment schedule for the miR-33 experiments.

(**a**) Spread of lentiviruses along the dorso-ventral hippocampus as revealed by immunohistochemistry with anti-GFP antibodies. (**b**) Treatment schedule for miR-33 overexpression with LV-miR-33. (**c**) Treatment schedule for miR-33 inhibition with miR-33-LNA.

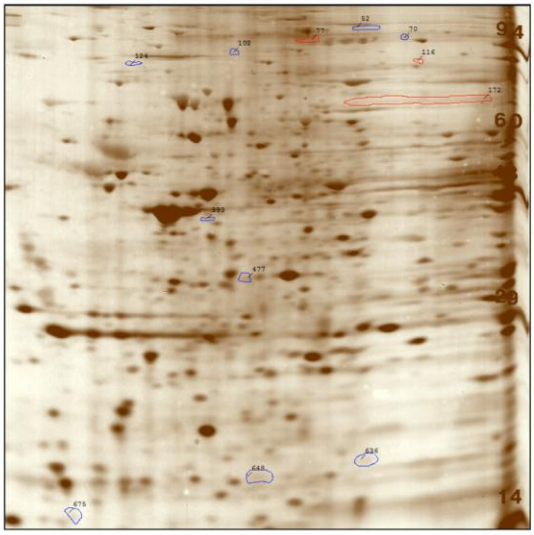


Supplementary Figure 7

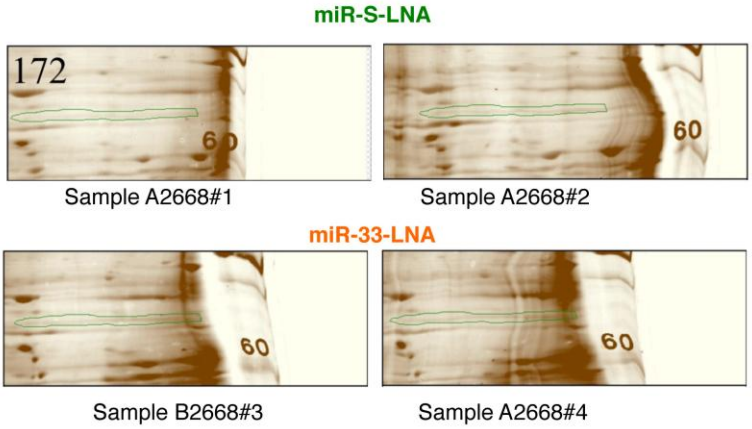
miR-33 manipulations affects the level of GABA-related proteins but not the level of NMDAR and protein kinases typically required for fear conditioning.

(**a**) Immunoblots showing the effect of miR-33 overexpression and (**b**) miR-33 inhibition on the levels of GABRA4, GABRB2, KCC2 and Syn2a,b (quantification shown in **Figs. 4b,c,e**). (**c**) miR-33 overexpression did not affect the level of the main NMDAR subunits NR1, NR2A, or NR2B (5 samples/protein/treatment; $F_{2,24} = 2.116$, $P = 0.115$) or (**d**) protein kinases cAMP-dependent protein kinase (PKA), calcium and calmodulin-regulated kinase II (CaMKII), or extracellular signal-regulated kinases 1/2 (Erk-1/2) (5 samples/protein/treatment; $F_{2,24} = 0.31$, $P = 0.861$).

a



b



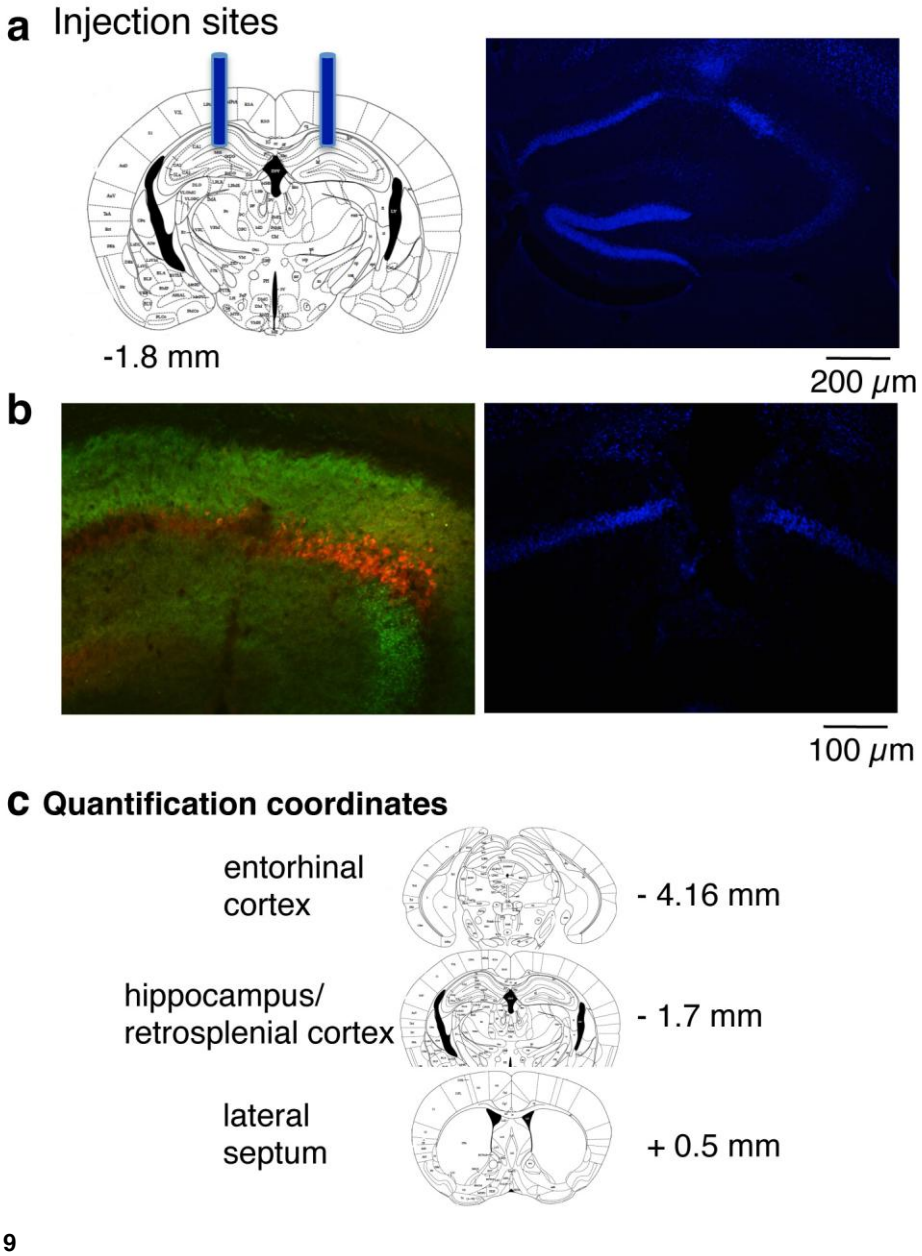
c

Spot #	pI	MW	Sample A 2688 #1 Spot %	Sample A 2688 #2 Spot %	Average Sample A Spot %	Sample B 2688 #3 Spot %	Sample B 2688 #4 Spot %	Average Sample B Spot %	Sample A vs Sample B Difference	Sample A vs Sample B T-test (p)
102 gelsolin	6.3	92131	0.011	0.010	0.010	0.005	0.005	0.005	2.0	0.009
393 creatine kinase B-type	6.1	42939	0.009	0.009	0.009	0.005	0.005	0.005	1.7	0.001
477 malate dehydrogenase	6.4	34037	0.024	0.025	0.024	0.012	0.013	0.013	1.9	0.007
636 alpha-crystallin B chain	7.0	15875	0.052	0.055	0.054	0.029	0.033	0.031	1.7	0.010
675 tubulin beta-2A chain	5.3	13519	0.037	0.042	0.040	0.013	0.021	0.017	2.3	0.040
77 Dynamin	6.7	109011	0.009	0.010	0.010	0.022	0.021	0.022	-2.2	0.003
172 Synapsin-2	7.4	72853	0.274	0.358	0.316	0.562	0.656	0.609	-1.9	0.043

Supplementary Figure 8

Proteomic analyses of hippocampal protein samples after miR-S-LNA or miR-33-LNA treatment.

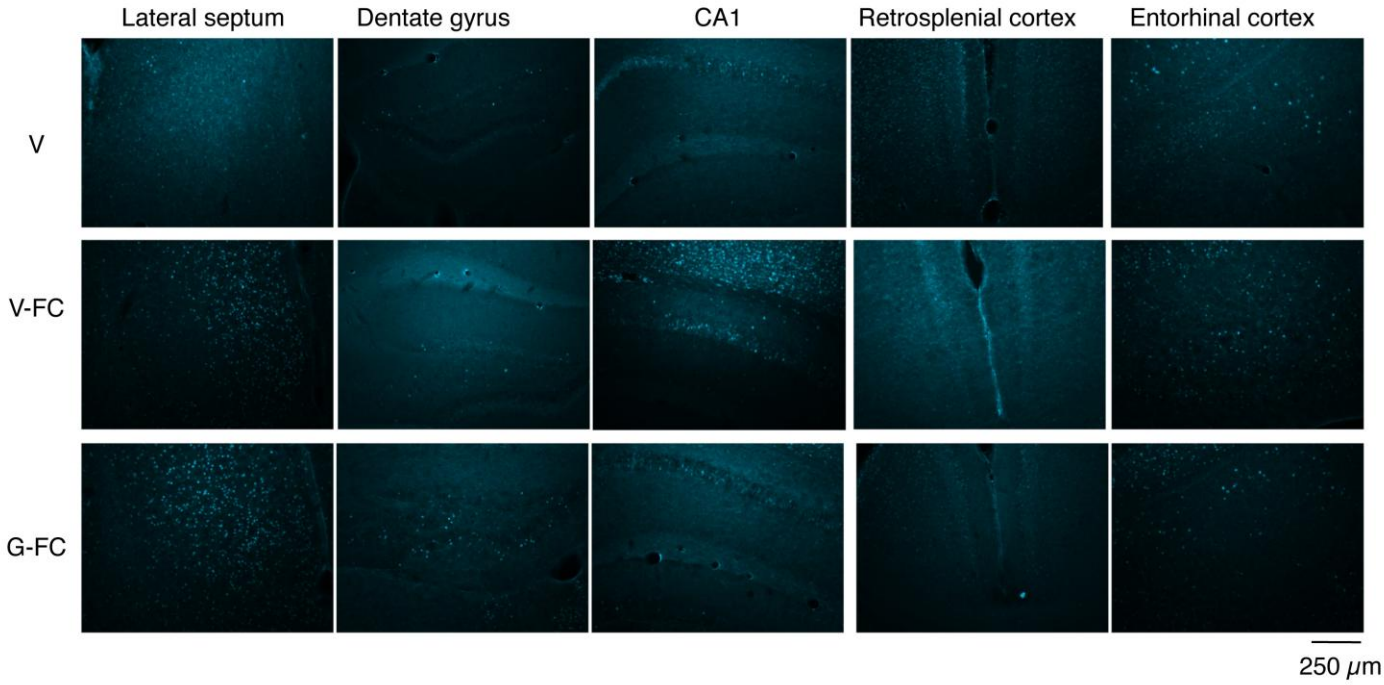
(**a**) 2D Gel Difference Image of averaged Sample A (vehicle-injected group) vs averaged Sample B (gaboxadol-injected group). Polypeptide spots increased in Sample A vs Sample B are outlined in Blue, while spots decreased in Sample A vs Sample B are outlined in Red. (**b**) The spot later identified as synapsin-2. (**c**) Identification of differentially produced proteins by mass spectrophotometry.



Supplementary Figure 9

Coordinates for infusions and immediate early gene response quantification.

(**a**) Hippocampal coordinates used for infusion of drugs, viruses, and microRNAs (left), and an example of cannula placement (right). (**b**) Higher magnification showing cannula traces in the CA1 subfield of mice injected with SynaptoTag followed by gaboxadol in images showing synapsin (green) and mCherry (red) (left) or EGR-1 (blue, right). (**c**) Coordinates used for quantification of EGR-1 and cFos immunostaining.



Supplementary Figure 10

cFos responses after fear conditioning with gaboxadol.

Individual photomicrographs of average cFos (pseudocolored green) immunostaining in different groups and brain areas. Significant changes were found in the lateral septum ($F_{2,8} = 5.232$, $P < 0.05$) *$P < 0.05$, **$P < 0.01$, when compared to the V group.