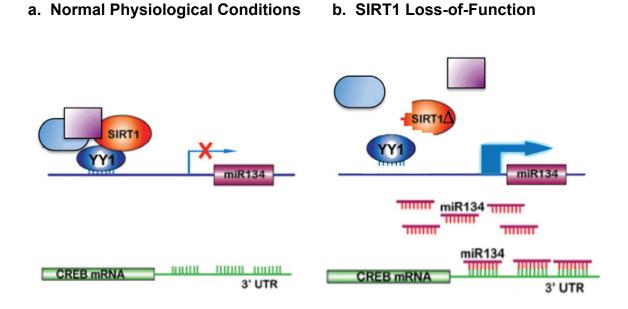
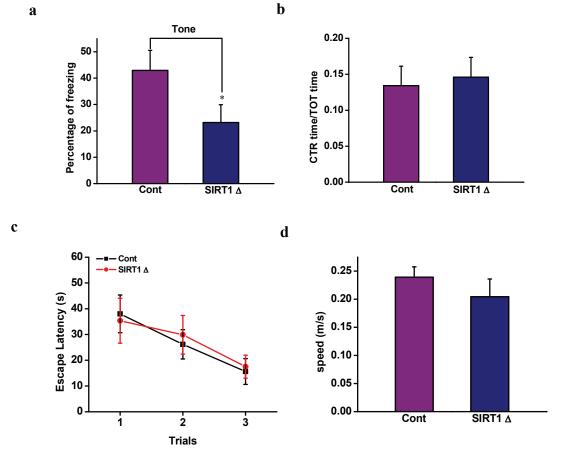
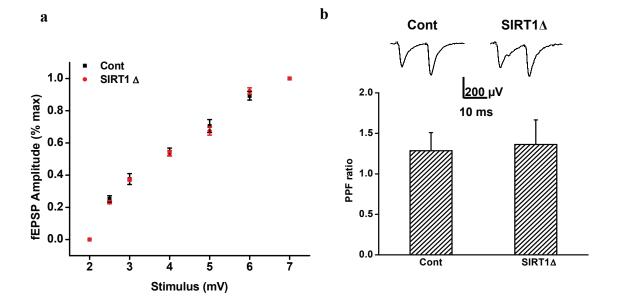
SUPPLEMENTARY INFORMATION

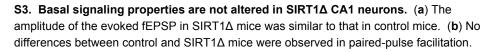


S1. Model for the role of SIRT1 in the regulation of memory and plasticity. (a) Our findings suggest that SIRT1 normally functions in cooperation with YY1, and potentially additional proteins, to restrict the expression of miR-134 and that, (b) upon SIRT1 loss-of-function, higher levels of miR-134 negatively regulate synaptic plasticity via the translational block of key plasticity proteins such as CREB.



S2. SIRT1 loss-of-function impairs learning and memory formation. (a) Tonedependent freezing behavior is reduced in SIRT1 Δ mice compared with control (Cont) mice (SIRT1 Δ , n = 13; Cont, n = 15, *p < 0.05). (b) Locomotor activity was not changed in SIRT1 Δ mice in comparison with controls for time spent in the center (p=0.6157). (c) SIRT1 Δ mice exhibit normal locomotor activity during the water maze test. Escape latencies of control and SIRT1 Δ mice in the visible platform water maze test. (d) The velocities of control and SIRT1 Δ mice during the probe test were similar.



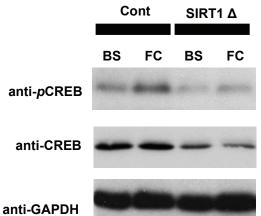


Cont after FC 2.0 Cont SIRT1∆ SIRT1∆ after FC 1.5 **Relative q-PCR signal** 1.0 0.5 0.0 BONF2 BONFA BONFI Cont SIRT1 **D** BS FC BS FC anti-pCREB

ChIP with CREB

a

b



S4. SIRT1 loss-of-function reduces the association of CREB with BDNF in the hippocampus. (a) Chromatin immunoprecipitation (ChIP) with an anti-CREB antibody followed by semi-quantitative PCR demonstrated a strong association of CREB with BDNF 1, 2 and 4 genes in floxed controls, but not in SIRT1A, hippocampal samples (n=3). Moreover, enrichment of BDNF1 and 4 was enhanced after 30 min fear conditioning stimulation in control mice, but not in SIRT1A mice. (b) The level of phosphorylated CREB was enhanced both in control and SIRT1Δ mice at 45 min after fear conditioning training. *p < 0.05, # = not significant.

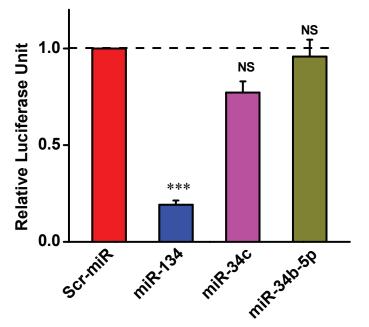
Cont	SIRT1 Δ	
-		microRNA
		mmu-miR-34b-5p mmu-miR-34c
		mmu-miR-126-5p mmu-miR-143
		mmu-miR-451
		mmu-miR-126-3p mmu-miR-144
		mmu-miR-137 mmu-miR-204
		mmu-miR-16
		mmu-miR-27a mmu-miR-153
		mmu-miR-32 mmu-miR-23a
		mmu-miR-23a mmu-miR-134
		mmu-miR-365 mmu-miR-708
		mmu-let-7a
		mmu-miR-21 mmu-miR-193
		mmu-miR-221 mmu-miR-29b
		mmu-miR-22
-		mmu-miR-31 mmu-miR-140
		mmu-miR-301a
		mmu-miR-350 mmu-miR-132
		mmu-miR-27b mmu-miR-135a
		mmu-miR-26b
		mmu-miR-142-5p mmu-miR-30e
		mmu-miR-503
		mmu-miR-29c mmu-miR-1
		mmu-miR-136 mmu-miR-29a
		mmu-miR-30b
		mmu-miR-378 mmu-miR-128
		mmu-miR-30a
		mmu-miR-342 mmu-miR-30c
		mmu-let-7d mmu-miR-28
		mmu-miR-668
		mmu-miR-720 mmu-miR-195
		mmu-miR-191
		mmu-miR-677 mmu-miR-101a
		mmu-miR-470
		mmu-miR-30d mmu-miR-411
		mmu-miR-125b-5p mmu-miR-434-3p
		mmu-let-7f
		mmu-miR-212 mmu-miR-34a
		mmu-miR-322 mmu-miR-125b-3p
		mmu-miR-99a
		mmu-miR-323-3p mmu-miR-425
		mmu-miR-770-3p
		mmu-miR-150 mmu-miR-325
		mmu-miR-369 mmu-miR-9
		mmu-miR-125b
		mmu-miR-106b mmu-miR-222
		mmu-miR-125a-5p mmu-miR-17
		mmu-miR-26a
		mmu-miR-218 mmu-miR-130a
		mmu-miR-15a
		mmu-miR-146a mmu-miR-186
		mmu-miR-138
		mmu-miR-24 mmu-miR-23b
		mmu-miR-872 mmu-miR-93
		mmu-miR-379
10		mmu-miR-99b mmu-miR-33
		mmu-miR-219
		mmu-miR-124a mmu-miR-185
		mmu-miR-19a mmu-miR-488
		mmu-miR-19b
		mmu-miR-338 mmu-miR-214
		mmu-miR-382
		mmu-miR-376a mmu-let-7c
1		mmu-let-7b mmu-miR-187
		mmu-miR-181a
		mmu-miR-127 mmu-miR-300
		mmu-miR-7a
		mmu-miR-145

S5. The expression of a number of brain-enriched microRNAs is altered in SIRT1Δ mice. A heat map diagram of the expression profile of highly enriched microRNAs in SIRT1Δ hippocampi and littermate control hippocampi was generated from a microRNA microarray (Exqion, Inc. miRCURY[™] LNA Array version 11.0). Each row represents a miRNA and the column represents the sample. The color scale illustrates the relative expression levels of a miRNA across samples: red color represents an expression level lower than the mean, green color represents expression level above than mean. Among the microRNAs differing in expression between the samples, miR-34b-5p, miR-126-5p, miR-144, miR-126-3p, miR-451, miR-34c, miR-153, miR-190, miR-32, miR-708, miR-23a, miR-16, miR-21, miR-134 miR301a and miR22 were upregulated by more than 25% in SIRT1Δ hippocampi compared with control, while miR-187, miR-467, miR-221 and miR-872 were downregulated by more than 25%.

a

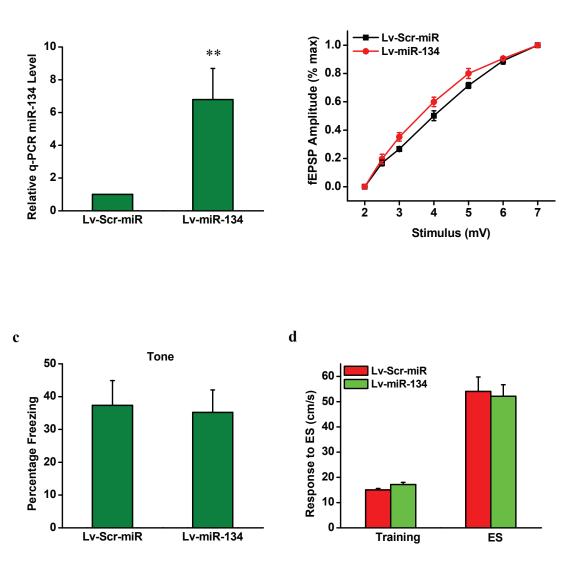
5'	CREB mRNA 3'				
UTR	EXON		UTR		
Mouse	1# GUUAC <mark>CAGUCAC</mark> UUA		2# AGUCACUGUUAU		
Rat Guinea Pig	GUUACCAGUCACUUA				
Rabbit human	GUUGGUAUUCACUUG GUUACUAGUCACUUG	AAGUUGGCUA	CAUAGUCACUG - L CA <mark>CAGUCAC</mark> UG - L	JAUGU	
Chimpanzee Rhesus	GUUACUAGUCACUUG GUUACUAGUCACUUG		CA cagucac ug - l Ca cagucac ug - l		
		3#			
Mouse Rat	UUUUUACACAGACAUUUAU UUUUUACACAGACAUUUAU				
Guinea Pig Rabbit	UUUUU · · · · AGACAUUUAU				
Human Chimpanzee	UUUUUACACAGACAUUUAU UUUUUACACAGACAUUUAU				
Rhesus	UUUUUACACAGACAUUUAU				

b



S6. The CREB 3'UTR contains three putative miRNA-134 binding sites and overexpression of miR-34c or miR-34b-5p did not affect CREB activity in cultured CAD cells. (a) Sequence alignment of miR-134 binding sites (predicted using the Miranda algorithm) in the CREB 3'UTR. (b) CREB activity was not altered after transfection of miR34c (p=0.32, n=3) or miR-34b-5p (p=0.62, n=3), two other microRNAs found to be upregulated in SIRT1 Δ mice. ***p < 0.001.

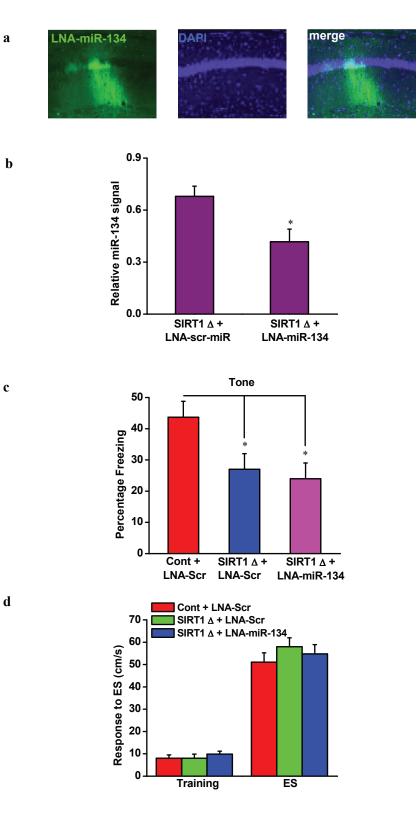
a



b

S7. Overexpression of miR-134 in the hippocampus does not affect tone-dependent fear conditioning or locomotion in mice. (a) Real-time PCR demonstrated upregulated miR-134 6 weeks after lentivirus expressing miR-134 (Lv-miR-134), or control virus (Lv-ScrmiR), was injected into the dorsal hippocampus. (b) The overexpression of miR-134 in CA1 had no effect on evoked fEPSP amplitude. (c) Six weeks after receiving hippocampal injections of Lv-miR-134, mice did not exhibit impaired freezing behavior during the tone memory test compared with Lv-Scr-miR-injected controls (Lv-miR-134, n = 14; Lv-Scr-miR, n = 12, p=0.6057, Student's *t*-test). Freezing activity is displayed as average ± SEM. (d)

Velocity of both groups during the training and the electric foot shock (I=0.8 mA). **p < 0.01.



S8. Injection of LNA-miR-134 downregulates miR-134 levels in the hippocampus of SIRT1 Δ mice. (a) Representative images showing the expression of fluorescently-labeled scrambled microRNA in area CA1 of SIRT1 Δ mice 2 days after injection. (b) Real-time PCR demonstrated decreased miR-134 levels in the SIRT1 Δ hippocampus 2-3 days after LNA-miR-134 injection compared to LNA-scr-miR injection. ***p < 0.001, Student's *t*-test. (c) The impaired tone-dependent memory in the SIRT1 Δ mice was not rescued by LNA-miR-134 injection into hippocampus. *p < 0.05. (d) The response to ES of all groups during the training and the electric foot shock (I=0.8 mA).