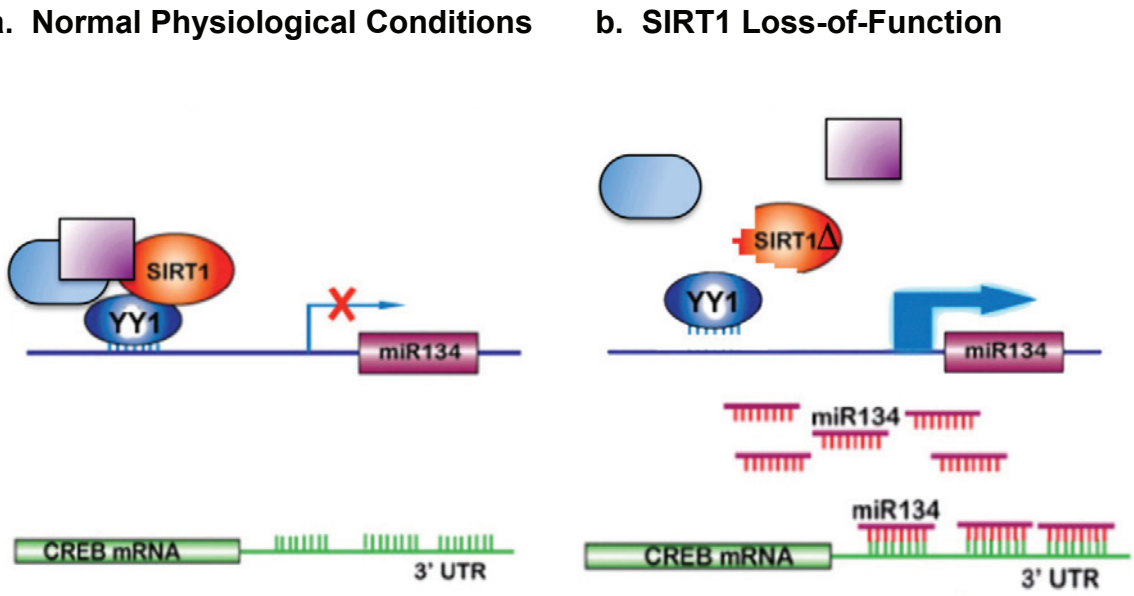
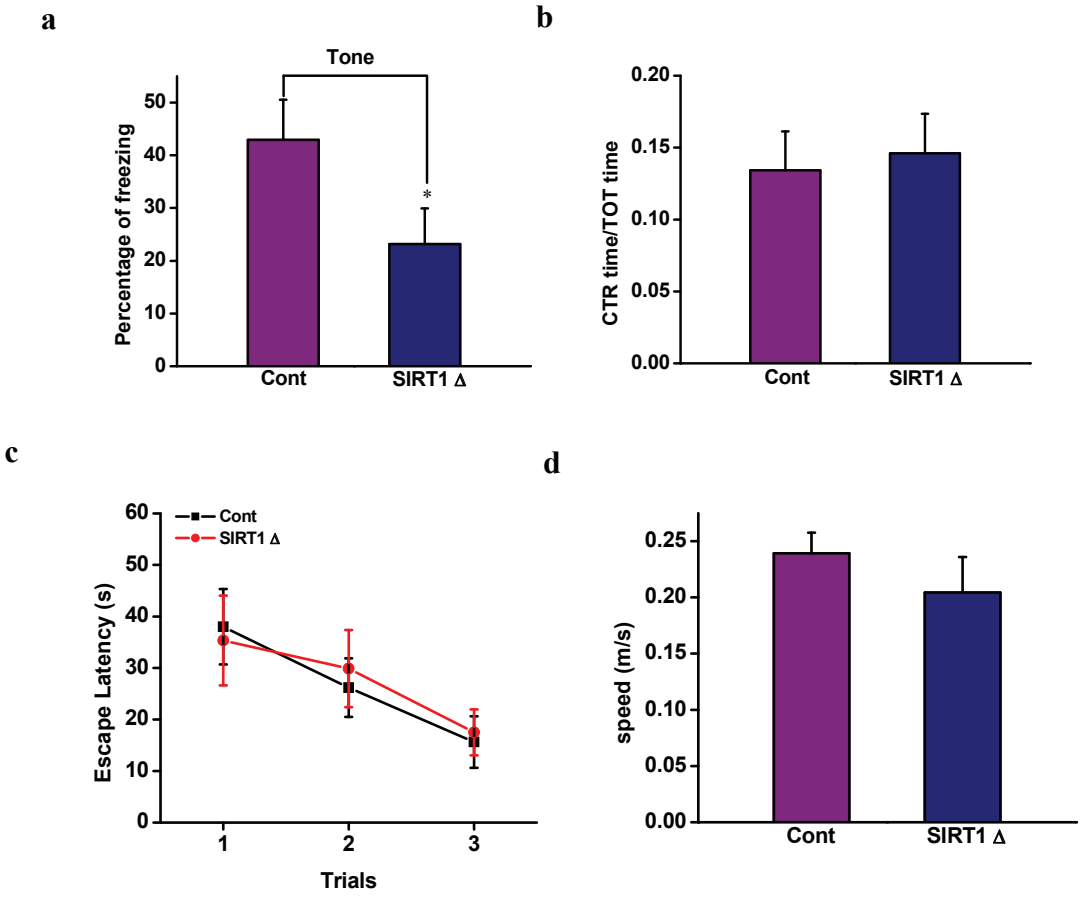
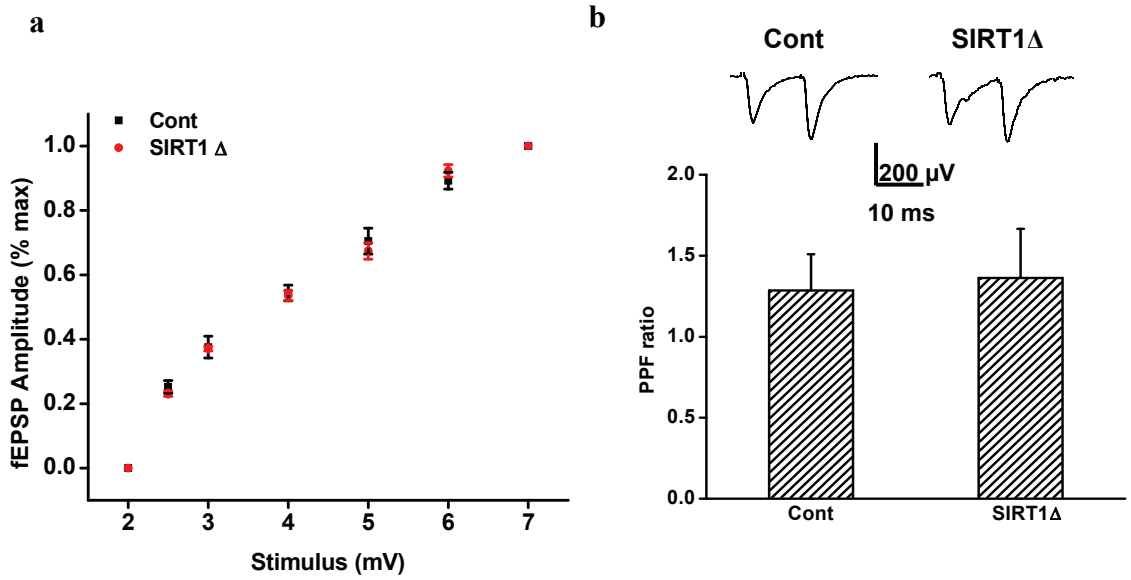
SUPPLEMENTARY INFORMATION



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.

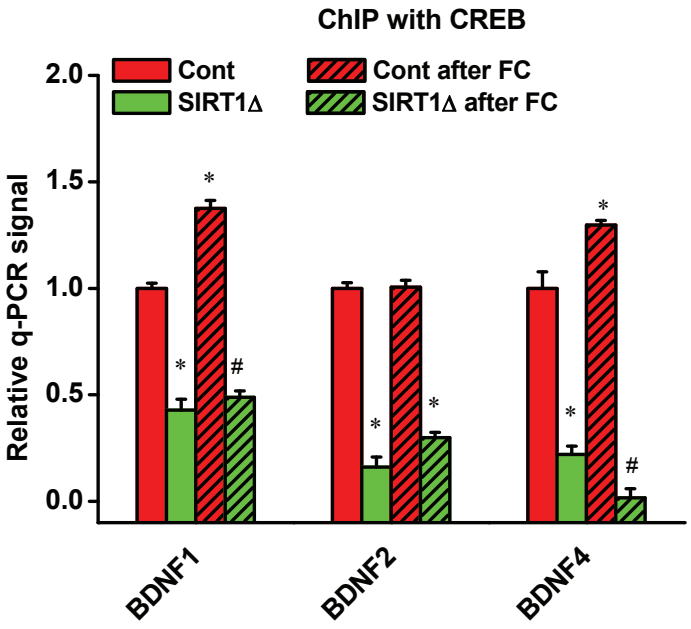


S2. SIRT1 loss-of-function impairs learning and memory formation. (**a**) Tone-dependent freezing behavior is reduced in SIRT1Δ mice compared with control (Cont) mice (SIRT1Δ, n = 13; Cont, n = 15, *$p < 0.05$). (**b**) Locomotor activity was not changed in SIRT1Δ mice in comparison with controls for time spent in the center (p=0.6157). (**c**) SIRT1Δ mice exhibit normal locomotor activity during the water maze test. Escape latencies of control and SIRT1Δ mice in the visible platform water maze test. (**d**) The velocities of control and SIRT1Δ mice during the probe test were similar.

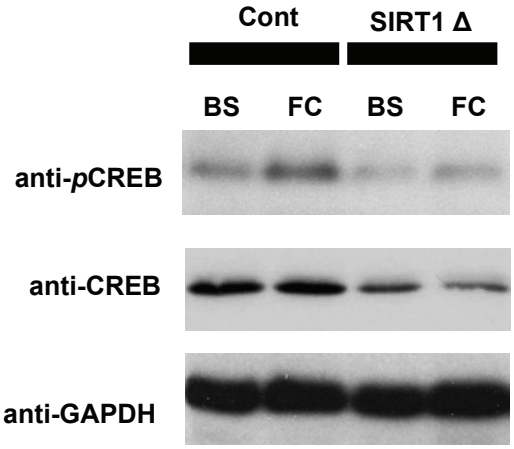


S3. Basal signaling properties are not altered in SIRT1Δ CA1 neurons. (**a**) The amplitude of the evoked fEPSP in SIRT1Δ mice was similar to that in control mice. (**b**) No differences between control and SIRT1Δ mice were observed in paired-pulse facilitation.

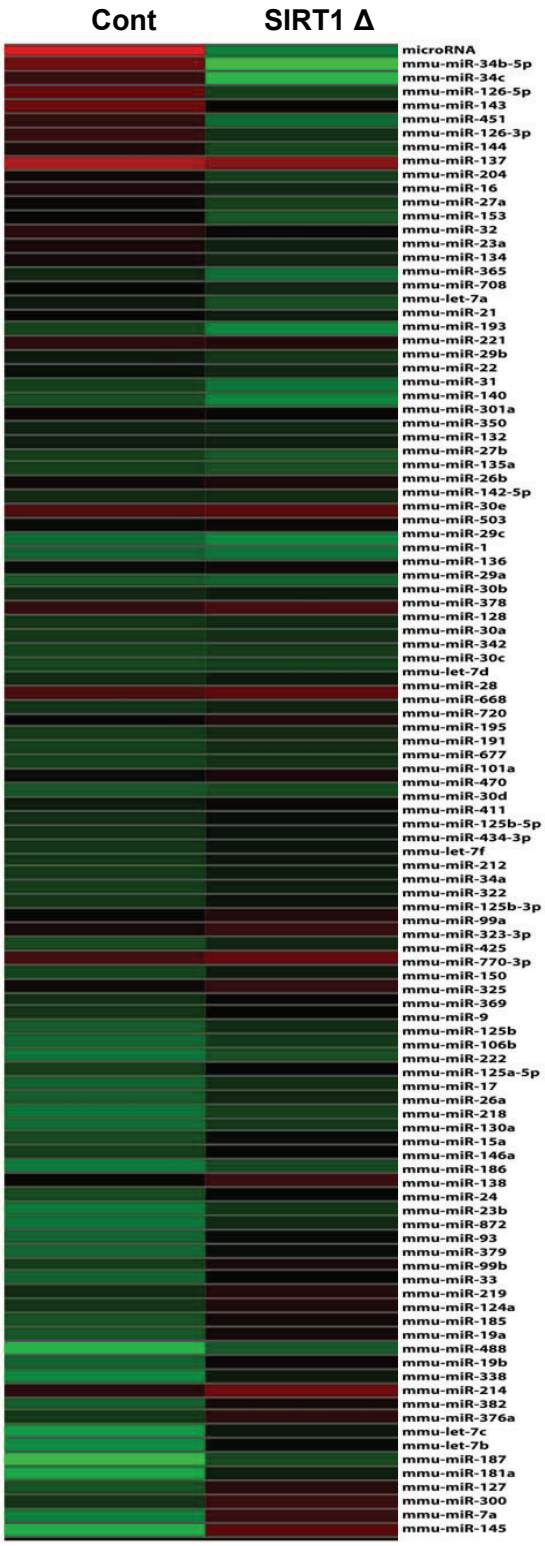
a



b

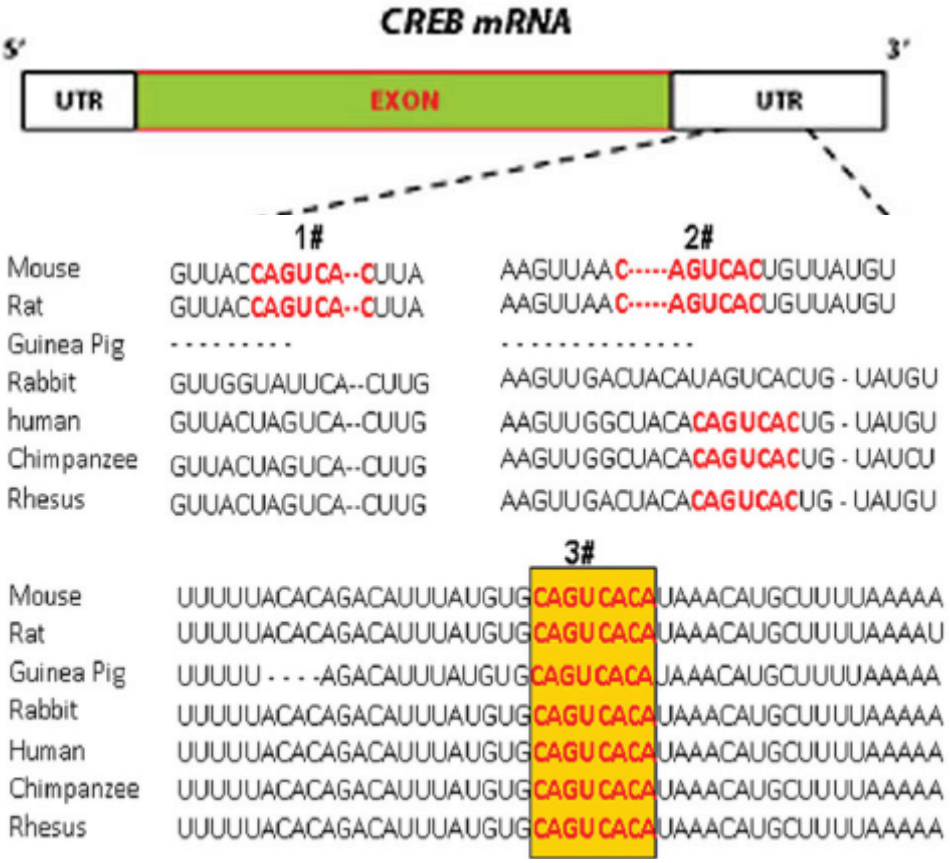


S4. SIRT1 loss-of-function reduces the association of CREB with BDNF in the hippocampus. (**a**) Chromatin immunoprecipitation (ChIP) with an anti-CREB antibody followed by semi-quantitative PCR demonstrated a strong association of CREB with BDNF 1, 2 and 4 genes in floxed controls, but not in SIRT1Δ,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

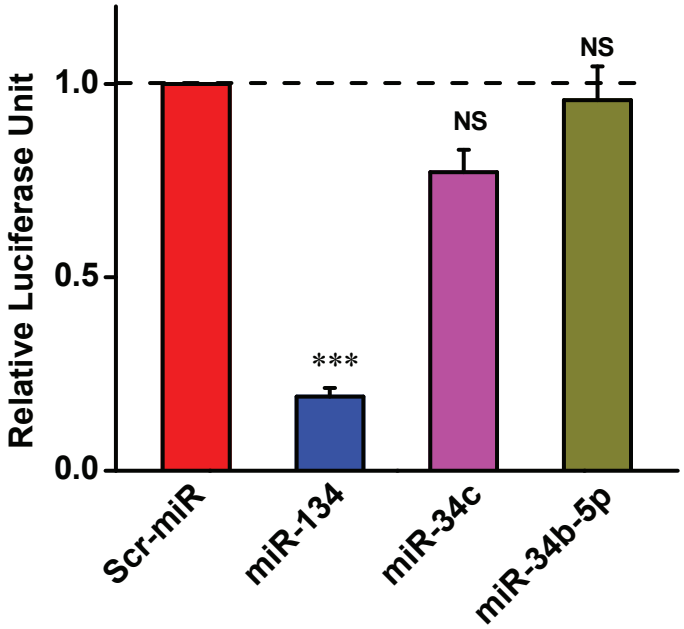


S5. The expression of a number of brain-enriched microRNAs is altered in SIRT1Δ mice. A heat map diagram of the expression profile of highly enriched microRNAs in SIRT1Δ hippocampi and littermate control hippocampi was generated from a microRNA microarray (Exqion, Inc. miRCURY™ LNA Array version 11.0). 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%.

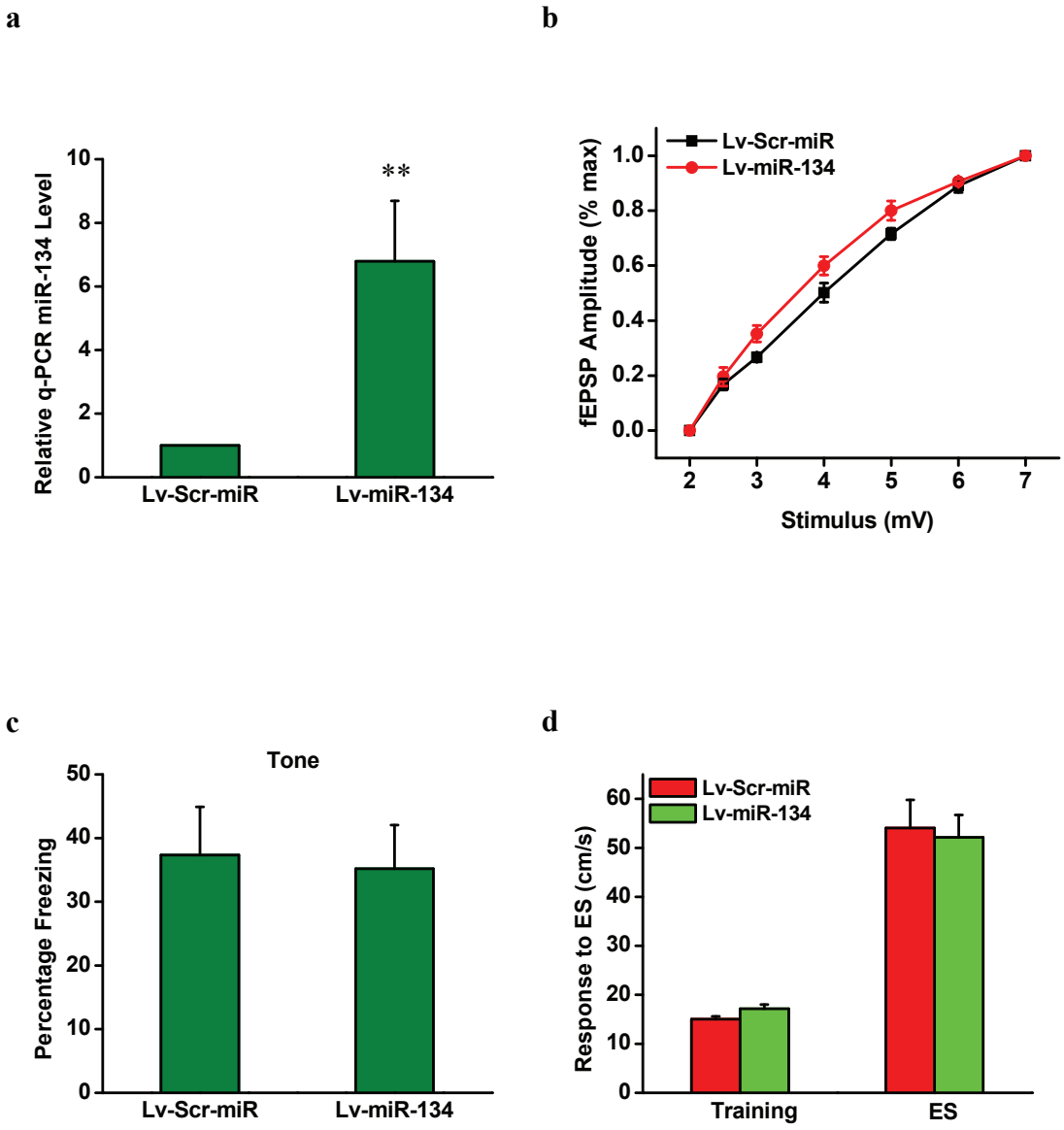
a



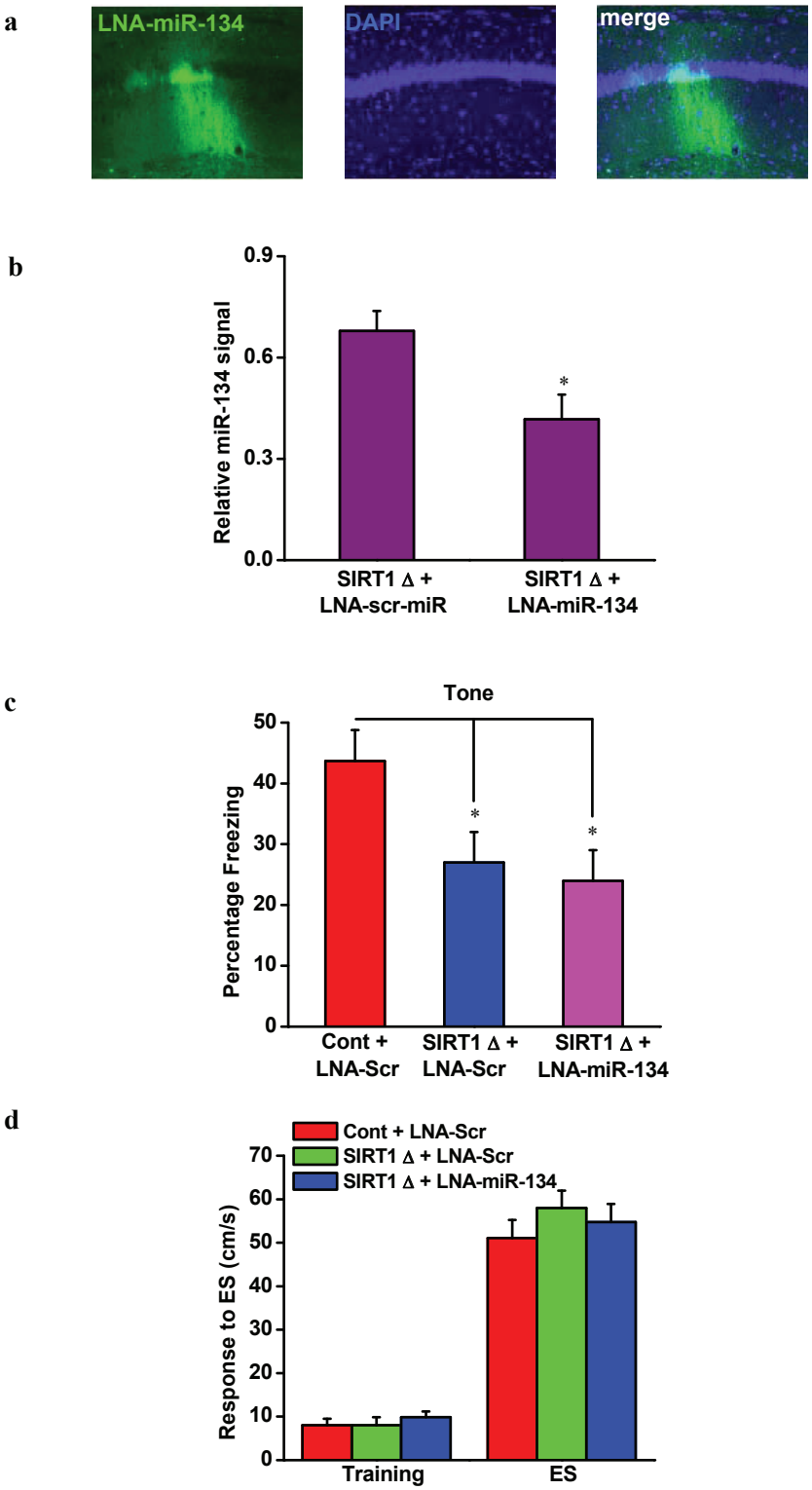
b



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 SIRT1Δ mice. ***$p < 0.001$.



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-Scr-miR), 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 SIRT1Δ mice. (**a**) Representative images showing the expression of fluorescently-labeled scrambled microRNA in area CA1 of SIRT1Δ mice 2 days after injection. (**b**) Real-time PCR demonstrated decreased miR-134 levels in the SIRT1Δ 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 SIRT1Δ 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).