Supplemental Methods

Inhibition of Erk activity by U0126. To establish the dependence of fear extinction on Erk signaling, mice were injected into the dorsal hippocampus with 1 µg/0.25 µl/site of U0126, an inhibitor of Erk phosphorylation by the mitogen activated and extracellular signal-regulated kinase. The drug was injected i.h. immediately after every extinction test.

Effect of stronger training on fear conditioning and extinction. To increase the strength of the conditioning memory, we employed: a. a group exposed to three 2-s shocks of 0.7 mA at 1-min intervals during a 3-min contextual exposure; or b. a group exposed to a single 2-s, 1.5 mA footshock at the end of a 3 min contextual exposure.

Legends for Supplemental Figures

Figure S1. Behavioral and molecular responses of cFos-LacZ mice to conditioning and extinction. **A**, cFos-LacZ mice did not differ from their wild type littermates or C57BL/6 mice in their ability to acquire and extinguish contextual fear. **B**, Injections of FDG did not affect acquisition and extinction of contextual fear in cFos-LacZ mice. **C**, I.h. injections of U0126 (0.5 µg/0.25 nl/site) immediatelly after individual extinction tests prevented extinction in cFos-LacZ mice (*$p < 0.05$, **$p < 0.01$ vs vehicle). **D**, The number of cFos and **E**, pErk cells in response to conditioning and extinction, respectively, did not differ among cFos-LacZ, wild type and C57BL/6 mice. The number of FDG+ cells was lower but not statistically significant.

Figure S2. Differences in FDG and pErk co-localization between the hippocampal CA1 area and parietal cortex. **A**, Hippocampus. Left column: pErk (top), FDG (middle) and composite (bottom) micrographs of CA1. Right: upper and lower rows: lack of detectable co-localization of FDG and pErk signals. Clear nuclear FDG signals and nuclear FDG signals overlayed by pErk+ passing fibers are marked. **B**, Parietal cortex. Left column: pErk (top), FDG (middle) and composite (bottom) micrographs of the parietal cortex adjacent to the hippocampus shown above. Right: upper and lower rows: clear co-localization of FDG and pErk signals. Labels: dashed squares, areas in the low magnification micrographs (left) selected to capture the high magnification micrographs (right); green stars, FDG+ cells; yellow stars, pErk+/FDG+ cells; yellow arrow, overlays of pErk+ fibres and FGD puncta.

Figure S3. Levels of cFos and pErk after stronger training conditions. **A**, Mice trained with one or three training trials (3 footshocks spaced beween 1 min contextual exposures) exhibited similar levels of freezing after training and during extinction ($F_{4,70} = 0.105$, $p = 0.98$). **B**, Mice trained with a stronger shock (one 1.5 mA footshock delivered after a 3-min contextual exposure) exhibited similar levels of freezing after training but impaired extinction ($F_{4,70} = 3.91$, $p < 0.05$) when compared to the 0.7 mA group, indicating stronger fear memory. **C**, Mice trained with 1.5mA footshock had similar cFos levels in the hippocampus after training ($t_8 = 0.27$, $p = 0.73$) and reduced pErk levels on E5 ($t_8 = 4.11$, $p < 0.05$) when compared to the 0.7 mA group. (*$p < 0.05$).