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Supplementary Materials for

Fear extinction requires ASIC1a-dependent regulation of hippocampal-prefrontal correlates

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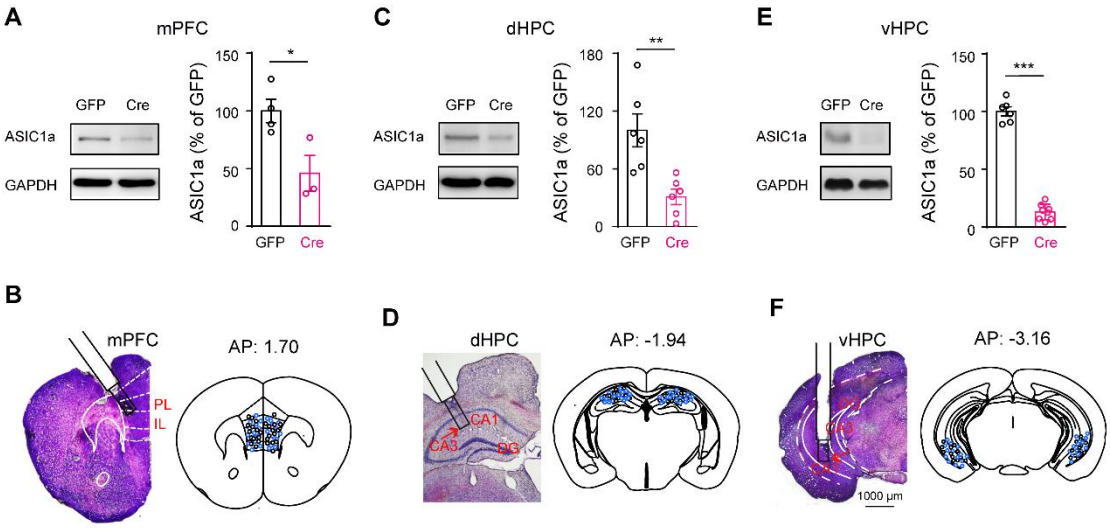

Fig. S1. Validation of knockdown of ASIC1a and cannula implantation in different brain regions. (**A**, **C**, **E**) Western blots showing ASIC1a protein levels in mPFC (**A**), dHPC (**C**), or vHPC (**E**) of AAV-Syn-GFP (GFP)- and AAV-Syn-Cre-GFP (Cre)-injected *Asic1a*$^{flox/flox}$ mice. *Left*, representative blots. *Right*, summaries (mean ± s.e.m.). (A) mPFC: GFP, n = 4; Cre, n = 3; (C) dHPC: n = 6 per group; (E) vHPC: GFP, n = 6; Cre, n = 8. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, GFP versus Cre, unpaired Student's *t*-test. (**B**, **D**, **F**) Verification of cannula implantation in mPFC (B), dHPC (D), and vHPC (F) performed using C57BL/6J. The site of drug infusion was verified by Nissl staining. The diagrams indicate sites of saline (*black*) or PcTX1 (*blue*) infusion for all samples.

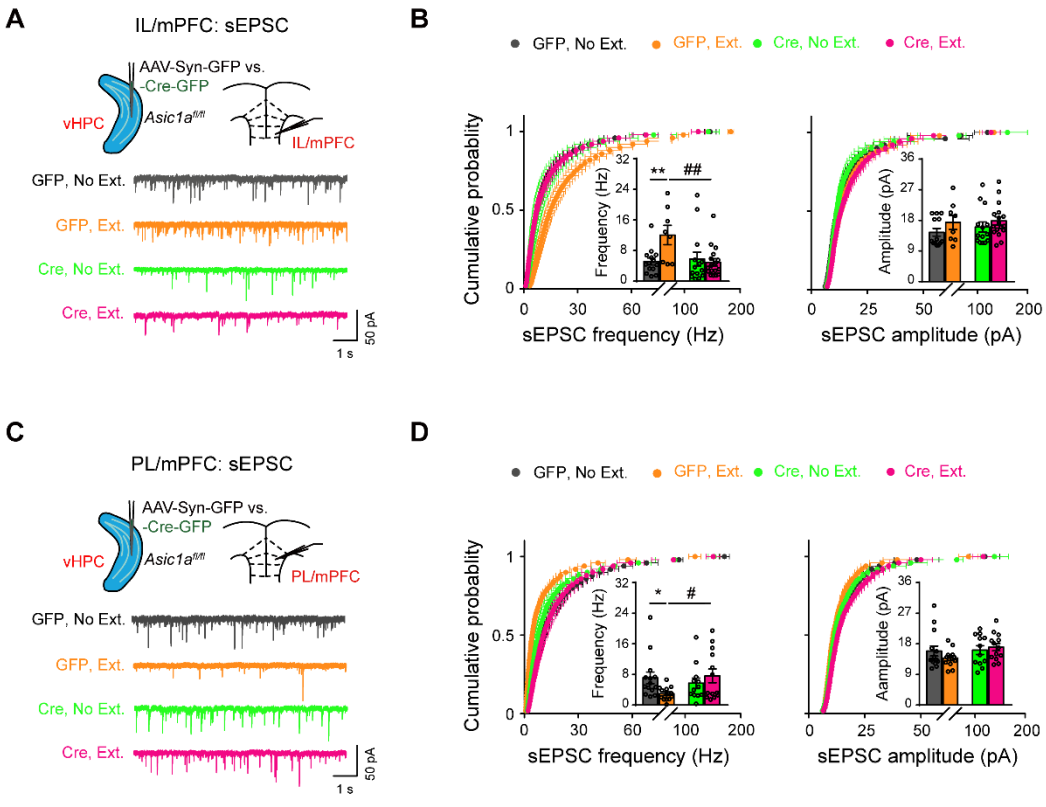

Fig. S2. ASIC1a in vHPC is involved in extinction learning–driven changes of sEPSC (as global synaptic inputs) in mPFC neurons. (**A**, **C**) Experimental schemes (*upper*) and representative traces of sEPSCs (*bottom*) in the neurons of IL/mPFC (A) or PL/mPFC (C). (**B**, **D**) Cumulative distribution plots for sEPSC frequency (*left*) and amplitudes (*right*). Inset show values for individual neurons and the summary. (B) IL-mPFC, GFP, No Ext., n = 14 neurons of 8 mice; GFP, Ext., n = 8 neurons of 4 mice; Cre, No Ext., n = 14 neurons of 8 mice; Cre, Ext., n = 17 neurons of 8 mice. (D) PL-mPFC, GFP, No Ext., n = 9 neurons of 7 mice; GFP, Ext., n = 11 neurons of 8 mice; Cre, No Ext., n = 16 neurons of 8 mice; Cre, Ext., n = 12 neurons of 7 mice. **P* < 0.05, ***P* < 0.01, No Ext. versus Ext.; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, unpaired Student's *t*-test.

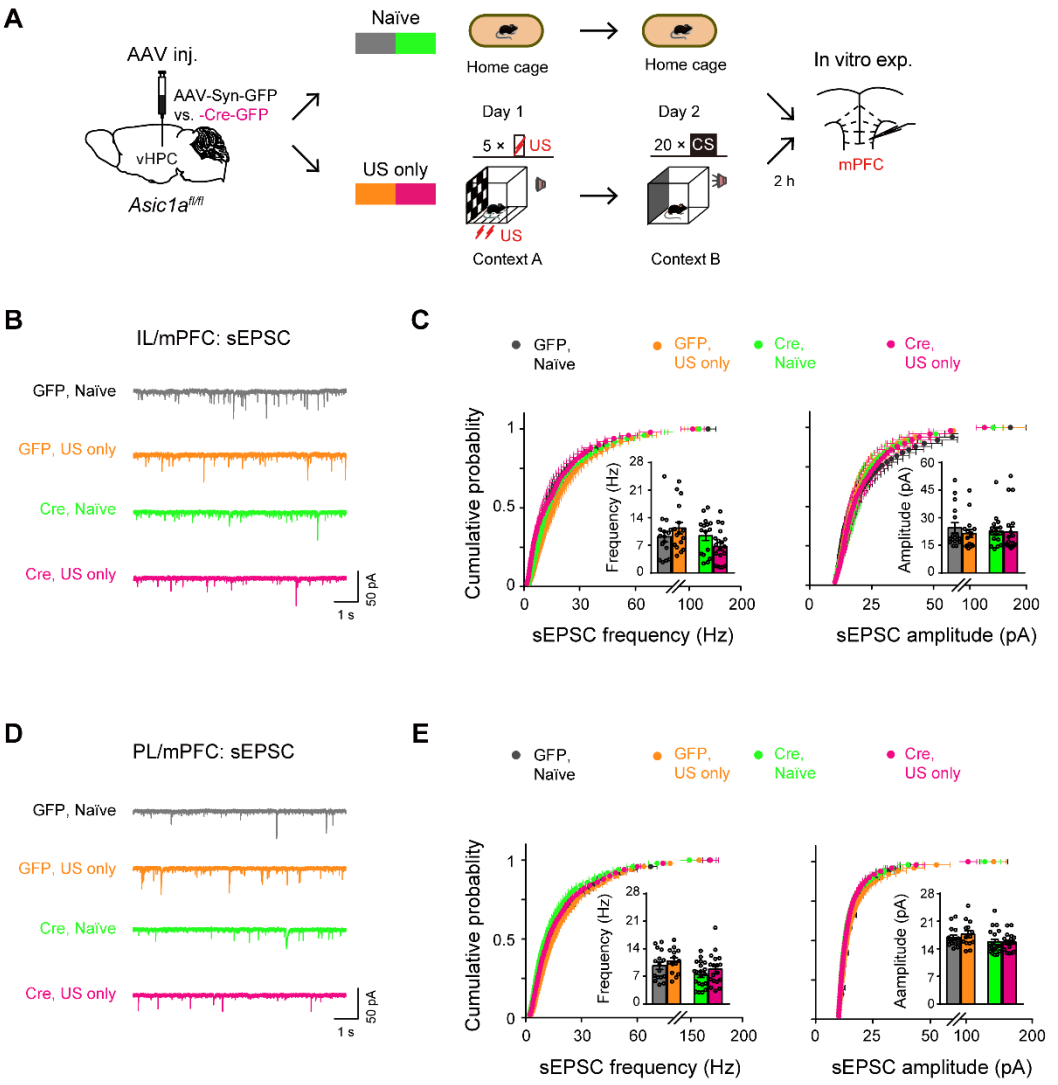

Fig. S3. US only does not alter sEPSC in mPFC neurons. (**A**) Experimental schemes. *Asic1a*$^{flox/flox}$ mice were injected bilaterally at vHPC with AAV-Syn-Cre-GFP (Cre) for *Asic1a* gene inactivation or AAV-Syn-GFP (GFP) as the control. After 6-8 weeks, the mice were used without treatment (naïve) or subject to five footshocks (US; 0.5 mA, 2 s) without cue tone pairing in context A (US only). The US only animals were further exposed to 20 pure tones (20 × CS) on the second day, 2 h before the preparation of brain slices containing the mPFC region. (**B**, **D**) Representative traces of sEPSCs in the neurons of IL/mPFC (B) or PL/mPFC (D) at a holding potential of –70 mV. (**C**, **E**) Cumulative distribution plots for sEPSC frequency (*left*) and amplitudes (*right*). Inset show values for individual neurons and the summary. (C) IL-mPFC, GFP, Naïve, n = 16 neurons of 3 mice; GFP, US only, n = 16 neurons of 3

mice; Cre, Naïve, n = 16 neurons of 3 mice; Cre, US only, n = 19 neurons of 3 mice. (E) PL-mPFC, GFP, Naïve, n = 16 neurons of 3 mice; GFP, US only, n = 14 neurons of 3 mice; Cre, Naïve, n = 20 neurons of 3 mice; Cre, US only, n = 18 neurons of 3 mice.

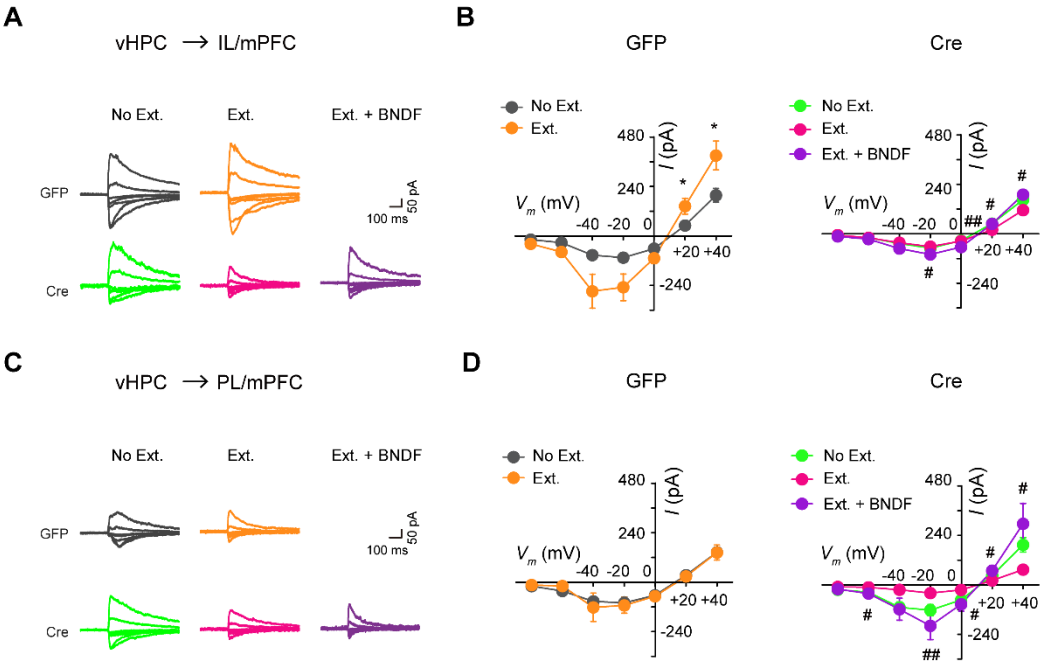

Fig. S4. Current-voltage relationships of NMDAR-mediated synaptic currents of different vHPC→mPFC projections. Animals were treated and brain slides were prepared as in Fig. 2. Shown are representative traces (**A**, **C**) and statistic results (**B**, **D**). (B) vHPC → IL/mPFC, GFP, No Ext., n = 8 neurons of 6 mice; GFP, Ext., n = 9 neurons of 7 mice; Cre, No Ext., n = 13 neurons of 8 mice; Cre, Ext., n = 12 neurons of 7 mice; Cre, Ext. + BDNF, n = 26 neurons of 9 mice. **P* < 0.05, for Ext. versus No Ext. of GFP mice. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, for Ext. versus Ext. + BDNF of Cre mice. (D) vHPC → PL/mPFC, GFP, No Ext., n = 13 neurons of 9 mice; GFP, Ext., n = 9 neurons of 7 mice; Cre, No Ext., n = 11 neurons of 6 mice; Cre, Ext., n = 8 neurons of 8 mice; Cre, Ext. + BDNF, n = 4 neurons of 3 mice. $^{\#}P < 0.05$ for Ext. versus Ext. + BDNF of Cre mice.

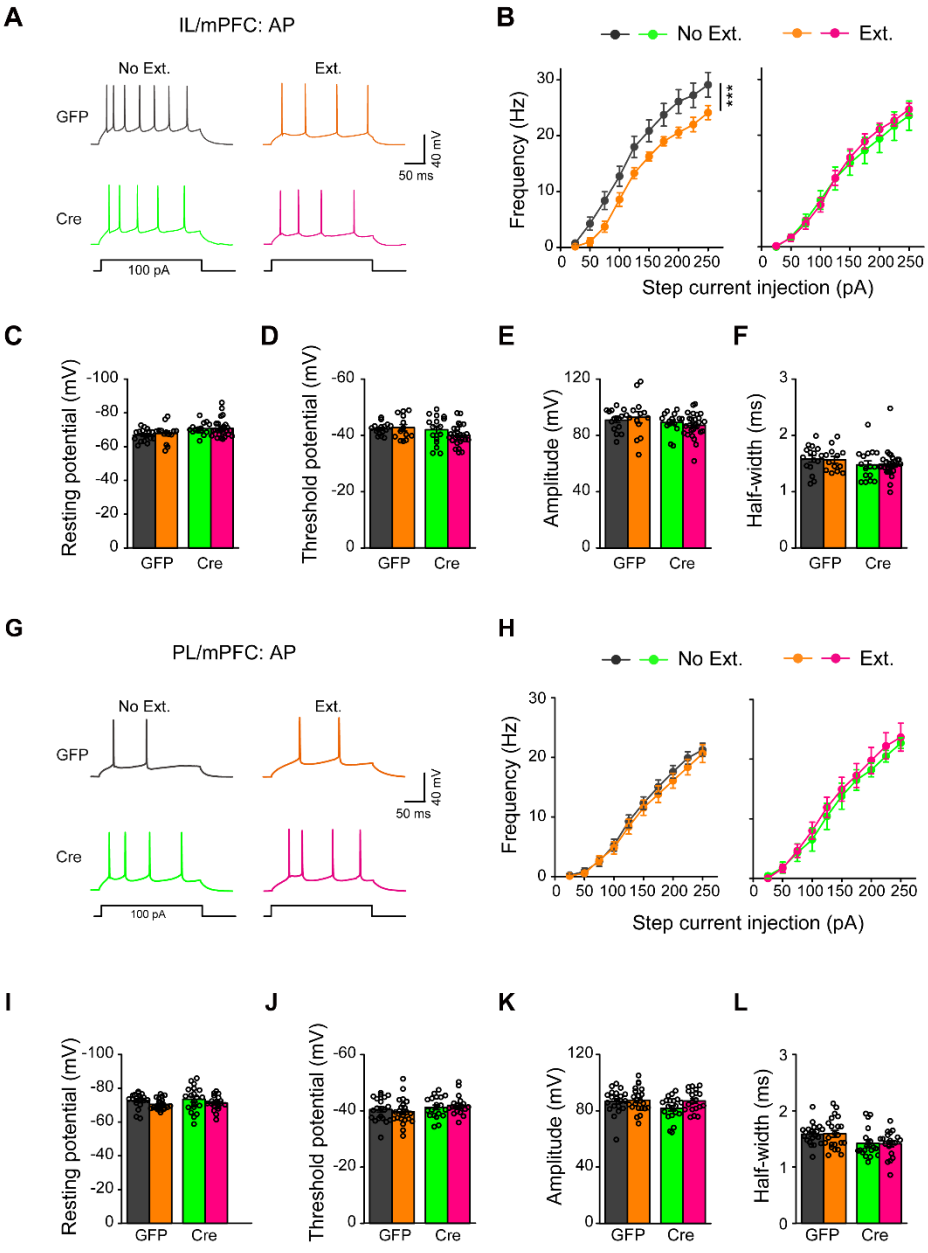

Fig. S5. ASIC1a-dependent effects of fear extinction training on the action potential firing of pyramidal neurons in mPFC. (**A**, **G**) Representative traces showing voltage responses of pyramidal neurons in layer II-III of IL/mPFC (A) or PL/PFC (G) to 500 ms step injection of current of +100 pA. (**B**, **H**) The frequency of action-potential discharge evoked by various step current intensity (0–250 pA, 500 ms). (B) IL/mPFC, *left*, GFP, two-way repeated measures ANOVA, main effect of behavior, $F_{1,298} = 39.415$, $P < 0.001$. *Right*, Cre, two-way repeated measures ANOVA, main effect of behavior, $F_{1,408} = 0.590$, $P = 0.443$. GFP, No Ext., n = 16 neurons of 7

mice; GFP, Ext., n = 14 neurons of 5 mice; Cre, No Ext., n = 16 neurons of 6 mice; Cre, Ext., n = 25 neurons of 9 mice. (H) PL/mPFC, *left*, GFP, two-way repeated measures ANOVA, main effect of behavior, $F_{1,408} = 2.328$, $P = 0.128$. *Right*, Cre, two-way repeated measures ANOVA, main effect of behavior, $F_{1,388} = 1.552$, $P = 0.214$. GFP, No Ext., n = 19 neurons of 7 mice; GFP, Ext., n = 22 neurons of 6 mice; Cre, No Ext., n = 20 neurons of 6 mice; Cre, Ext., n = 19 neurons of 7 mice. (**C** and **I**) Summary data for resting potential obtained from slice recording data set shown in (B and H). (**D** to **F**, **J** to **L**) Summary data for action potential threshold potential (D, J), amplitude (E, K), half-width (F, L), obtained from slice recording data set of the voltage responses evoked by 500 ms ramp injection of various current intensity (0–100, 0–200, 0–300, 0–400, 0–500 pA, respectively). (D to F), IL/mPFC: GFP, No Ext., n = 16 neurons of 7 mice; GFP, Ext., n = 13 neurons of 5 mice; Cre, No Ext., n = 16 neurons of 6 mice; Cre, Ext., n = 26 neurons of 9 mice. (J to L) PL/mPFC: GFP, No Ext., n = 20 neurons of 7 mice; GFP, Ext., n = 22 neurons of 8 mice; Cre, No Ext., n = 20 neurons of 6 mice; Cre, Ext., n = 19 neurons of 7 mice.

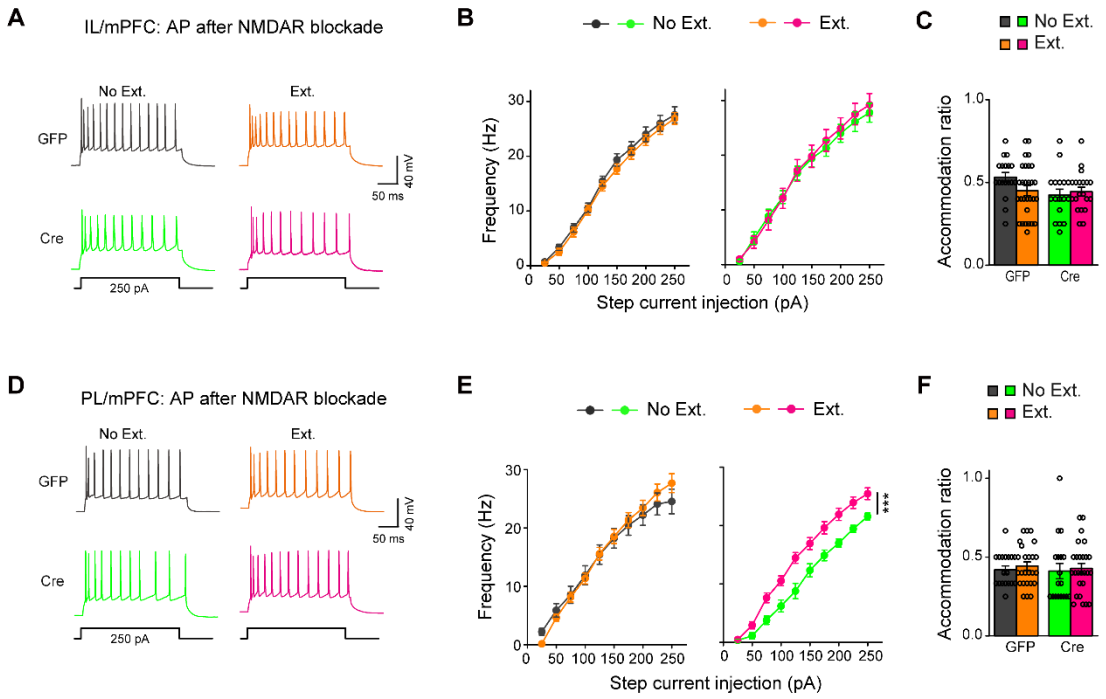

Fig. S6. Effects of NMDAR antagonism on action potential firing of pyramidal neurons in mPFC. Slice recordings were performed in manner similar with Fig. S5, but in the presence of NMDAR antagonist, D-APV (20 μM). (**A**, **G**) Representative traces showing voltage responses of pyramidal neurons in layer II-III of IL/mPFC (A) or PL/mPFC (D) to 500 ms step injection of current of +250 pA. (**B**, **E**) The frequency of action-potential discharge evoked by various step current intensity (0–250 pA, 500 ms). (B) IL/mPFC, *left*, GFP, two-way repeated measures ANOVA, main effect of behavior, $F_{1,468} = 3.450$, $P = 0.064$. *Right*, Cre, two-way repeated measures ANOVA, main effect of behavior, $F_{1,368} = 0.408$, $P = 0.523$. GFP, No Ext., n = 18 neurons of 5 mice; GFP, Ext., n = 29 neurons of 5 mice; Cre, No Ext., n = 17 neurons of 4 mice; Cre, Ext., n = 20 neurons of 3 mice. (E) PL/mPFC, *left*, GFP, two-way repeated measures ANOVA, main effect of behavior, $F_{1,428} = 0.335$, $P = 0.563$. *Right*, Cre, two-way repeated measures ANOVA, main effect of behavior, $F_{1,438} = 90.926$, $P < 0.001$. GFP, No Ext., n = 18 neurons of 5 mice; GFP, Ext., n = 25 neurons of 5 mice; Cre, No Ext., n = 18 neurons of 4 mice; Cre, Ext., n = 26 neurons of 3 mice. (**C**, **F**) Summary data for accommodation ratios of the action potential number obtained from slice recording data set shown in (B, E).

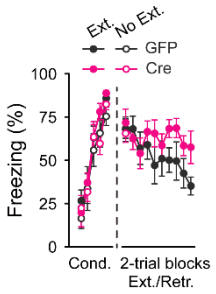

Fig. S7. Fear acquisition and extinction/retrieval of *Asic1a*$^{flox/flox}$ mice that received AAV-Syn-Cre-GFP and AAV-Syn-GFP injection at vHPC. Behavioral correlates of animals, of which vHPC tissues were used for RNA sequencing. The mice received either 2 trials (No Ext.) or 20 trials (Ext.) of CS alone in the test chamber before being sacrificed for vHPC dissection (for fear retrieval, one-way factorial ANOVA for average freezing during CS of 2 trials of No Ext. group and the first block of 2 trials of Ext. group, main effect of AAV, $F_{3,28} = 0.154$, $P = 0.926$; for fear extinction of AAV-Syn-GFP- and AAV-Syn-Cre-GFP-injected mice, $F_{1,14} = 1.764$, $P = 0.205$; n = 8 per group).

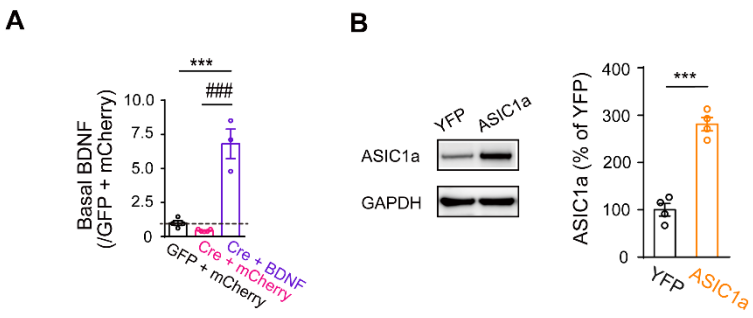

Fig. S8. Validation of AAV-mediated overexpression of BDNF or ASIC1a at vHPC. (**A**) Effects of injection of AAV-Syn-BDNF on basal BDNF expression in vHPC. Two-way repeated measures ANOVA, main effect of AAV, $F_{2,9} = 51.164$, $P < 0.001$ followed by post hoc Bonferroni, $P < 0.001$ for Cre + BDNF versus GFP + mCherry, $P < 0.001$ for Cre + BDNF versus Cre + mCherry, $P = 1.000$ for Cre + mCherry versus GFP + mCherry. GFP + mCherry, n = 4; Cre + mCherry, n = 5; Cre + BDNF, n = 3. ***$P < 0.001$, $^{###}P < 0.001$. (**B**) Effects of injection of AAV-Syn-ASIC1a on the ASIC1a expression in vHPC of C57 mice. Western blotting showing the expression of ASIC1a in vHPC of mice injected with AAV-Syn-YFP or AAV-Syn-ASIC1a-YFP. Shown are representative blot (*left*) and summary (individual data points and mean ± s.e.m., *right*). n = 4 per group. ***$P < 0.001$, unpaired Student's *t* test.

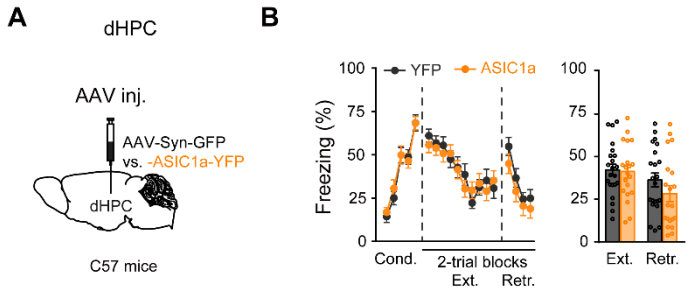

Fig. S9. Effects of *Asic1a* gene overexpression in dHPC on cued fear extinction. C57 mice were subject to AAV injection in dHPC (**A**) and tested 4 weeks later for fear acquisition, extinction learning and retrieval of extinction memory (**B**). (B) *Left*, two-way repeated measures ANOVA, main effect of AAV, fear conditioning, $F_{1,40}$ = 0.080, P = 0.778; fear extinction, $F_{1,40}$ = 0.054, P = 0.818; fear retrieval, $F_{1,40}$ = 1.323, P = 0.257. *Right*, average freezing during CS of all trials. YFP, n = 22; ASIC1a, n = 20.