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☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

Describe how sample size was determined.

We performed pilot experiments to determine sample sizes for measuring fear generalization.

2. Data exclusions

Describe any data exclusions.

Criteria were pre-established. Specifically: Mis-hits were excluded (see Methods). Additionally, Two mice were excluded from CA1-AA-day16 shNT and CA1-AC-day16 shNT group analysis because there were no tagged cells detectable in CA1 pyramidal layer. This is stated in the legend for Supplementary Fig 5.

3. Replication

Describe whether the experimental findings were reliably reproduced.

1. abLIM3 downregulation increases DGC recruitment of inhibition. This effect was replicated in multiple cohorts of mice and in two labs.
2. abLIM3 downregulation in DGCs decreases time-dependent fear generalization - This effect was observed in multiple groups of mice.
3. abLIM3 downregulation governs recactivation dynamics in hippocampal-cortical networks. This effect was replicated using multiple Tet-Tag reporters.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

Mice from Jackson or NIA or from breeders at MGH were randomly assigned to treatment groups. Age-matched mice arrived from JAX as 4/cage and were randomly assigned to shRNA vs. NTshRNA.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

1. All experiments were performed blinded to treatment. 2. Data was randomly selected by PI and scored by scientist in the lab not working on the experiment.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	Prism software was used for statistical analysis of data.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.	No restrictions

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	Primary antibodies: 1. c-fos: Rabbit anti c-fos, Calbiochem PC38, 1:10,000 – discontinued Different batches of rabbit, Santa Cruz SC52, 1:2,000 2. GFP: Rabbit anti-GFP, Life Technologies A11122, 1:500 3. GABA: Guinea-pig anti-GABA, Millipore AB175, 1:500 4. PV: mouse anti-PV, Millipore MAB1572, 1:2,000 5. SST: mouse anti-SST, Santa Cruz, sc-55565, 1:100 6. RGS14:mouse anti-RGS14, Biolegend N133/21, 1:400 7. β-gal: chicken anti-β-galactosidase, Abcam, 1:1000 8. VGLUT1:Guinea pig anti-VGLUT1, Synaptic Systems 135304, 1:3000 9. NeuN:Mouse anti-NeuN, Millipore MAB377 10. DCX:Goat anti- Doublecortin (DCX) sc-8066, Santa Cruz ,1:500 11. abLIM3: mouse anti-abLIM3, Abcam ab67721, 1:100 12. Bassoon: rabbit anti-bassoon, Abcam ab110426, 1:1000 13. ZO-1: rabbit anti-ZO-1 antibody, Invitrogen 402200, 1:500 Secondaries: Fluorescent-label-coupled secondary antibody (Jackson ImmunoResearch, 1:500).

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

HEK293T (ATCC)

b. Describe the method of cell line authentication used.

Commercially available and extensively validated, https://www.atcc.org/en/Products/All/CRL-3216.aspx

c. Report whether the cell lines were tested for mycoplasma contamination.

This cell line tested negative for mycoplasma contamination.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No commonly misidentified cell lines were used.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

All animals were handled and experiments were conducted in accordance with procedures approved by the Institutional Animal Care and Use Committees at the Massachusetts General Hospital and University of Washington, Seattle, in accordance with NIH guidelines. All mice were housed three-four per cage in a 12 hr (7:00 a.m. to 7:00 p.m.) light/dark colony room at 22°C–24°C with ad libitum access to food and water. Adult female C57Bl/6 mice (3-4 months old) were purchased from Jackson lab. Aged female C57Bl/6 mice (17-18 months old) were obtained from National Institute on Aging (NIA). The following mouse lines were obtained from Jackson Labs: cfos-tTA: tetO-taulacZ,tTA* (Stock No: 008344, Jackson labs), tetO-H2B-GFP (Stock No: 005104, Jackson labs), Col1a1-tetO-H2B-mCherry (Stock No: 014592, Jackson labs). TetTag mice were generated by crossing reporter mouse lines, tetO-H2B-GFP mouse line or tetO-H2B-mCherry mouse line, with mice that express tetracycline-trans-activator (tTA) protein under control of the c-fos promoter (Stock No: 008344, Jackson labs; outcrossed from tetO-lacZ, tTA*). c-fos-tTA mice also contain a transgene consisting of a c-fos promoter driving the expression of nuclear-localized 2-h half-life EGFP (shEGFP). All TetTag mice were maintained on a C57BL/6 background. Mice were bred and raised on doxycycline (dox) diet (40 mg kg−1 chow) to prevent any reporter expression prior to desired experimental labeling of ensembles. Dox diet was replaced with regular diet for 3 days to open the window for activity-dependent labeling of ensembles. Expression of reporter was shut off by administration of dox diet immediately following desired labeling window. PV-Cre mice (stock No: 017320, Jackson labs) were bred with Ai14: tdTomato reporter mouse line (stock No: 007914, Jackson labs) to generate mice for viral injection and in vitro electrophysiological whole cell recordings.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

The study did not involve human research participants