Supplementary Materials for

Ventral CA1 neurons store social memory

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Materials and Methods
Figs. S1 to S19

Other Supplementary Materials for this manuscript include the following:

Materials and Methods:

Subjects

All experiments were conducted in accordance with U.S. National Institutes of Health (NIH) guidelines and the Massachusetts Institute of Technology (MIT) Department of Comparative Medicine and Committee of Animal Care (CAC). All animals were socially housed in a 12 h (7am - 7pm) light/dark cycle, with food and water ad libitum. C57BL/6J wild-type mice were obtained from the Jackson Laboratory. c-fos:tTA transgenic mice were generated as described in (*17, 18*), by selecting mice carrying the c-fos:tTA transgene from offspring between TetTag (*18*) and C57BL/6J mice. c-fos:tTA without GFP transgenic mice (Stock No. 031756-MU) was obtained from Mutant Mouse Resource and Research Centers (MMRRC). c-fos:tTA and c-fos:tTA without GFP transgenic mice carry the tTA transgene with and without nuclear-localized enhanced GFP driven by a c-fos promoter, respectively. Both transgenic mouse lines were raised on a 40 mg/kg doxycycline (Dox) diet until the activated neuron labeling day. For dorsal CA1 Ca^{2+} imaging, we used previously developed Wfs1-Cre transgenic line (*19*). For histology of dorsal CA2 projection, we used previously developed Map3k15-Cre transgenic line (*28*). B6129SF1/J (Stock No: 101043) hybrid mice, which were the offspring of a cross between C57BL/6J females and 129S1/SvImJ males, were bred. We used B6129SF2, offspring of the B6129SF1/J mating pairs as subject-mice. All experiments were performed using male mice.

Generation of the Trpc4-Cre transgenic mouse line

A bacterial artificial chromosome (BAC) clone (RP24-67D9: 197 kb long) containing the *transient receptor potential channel 4* (*Trpc4*) gene was purchased from the BACPAC Resource Center. The nuclear localization sequence (NLS)-Cre-poly A-FRT-amp-FRT cassette was inserted into the translation initiation site located in the 2nd exon of Trpc4 using Red/ET recombination techniques. The FRT-amp-FRT selection marker gene cassette was further deleted by expressing yeast-derived recombinase (FLP) in the host *E. coli* cells. The resulting Trpc4-Cre BAC vector DNA was purified with NucleoBond BAC100 kit, linearized by *NotI* digestion, and fractionated on a Sepharose CL4b column

with an injection buffer (10 mM Tris-HCl pH7.4, 0.1 mM EDTA, 100 mM NaCl), and microinjected into fertilized C57BL/6J mouse eggs. The founder males were crossed with C57BL/6J females. The line used in this study was selected by characterizing Cre-mediated recombination patterns by crossing with *Gt(ROSA)26Sor*^{tm1Ito} (Rosa-NLS-LacZ) reporter mice (*32*).

Adeno-associated viruses (AAV)

AAV8-CamKIIa:eArchT3.0-EYFP was generated by and acquired from University of North Carolina (UNC) Vector Core, with a titer of 2.7*10^12 genome copies/ml. AAV9-hSyn:DIO-eArchT3.0-EYFP was generated by and acquired from University of Massachusetts Medical School (UMMS), with a titer of 2.5*10^13 genome copies/ml. AAV9-EF1a:DIO-EYFP (AV-9-27056) was generated by and acquired from University of Pennsylvania Vector Core, with a titer of 2.93*10^13 genome copies/ml. AAV9-EF1a:DIO-hChR2(H134R)-EYFP (AV-9-20298P) was generated by and acquired from University of Pennsylvania Vector Core, with a titer of 1.6*10^13 genome copies/ml. AAV5-Syn:DIO-GCaMP6f (AV-5-PV2819) was generated by and acquired from University of Pennsylvania Vector Core, with a titer of 5.9*10^12 genome copies/ml. pTRE:Fluorescent Timer (FT)-Slow was obtained from Addgene (Plasmid No. 31915) and pTRE:FT-Slow was cloned in AAV vector. AAV9-TRE:FT-Slow was packaged by ViGENE Bioscience, with a titer of 1.64*10^14 genome copies/ml. AAV9-TRE:ChR2-EYFP (2.0*10^13 genome copies/ml) and AAV9-TRE:EYFP (1.5*10^13 genome copies/ml) were generated by and acquired from the Gene Therapy Center and Vector Core at the University of Massachusetts Medical School, which were previously described (*17*). The plasmid of AAV5-TRE:histoneH2B-EGFP-2A-TVA receptor-2A-rabies glycoprotein (pAAV-TRE:H2B-EGFP) was obtained from Addgene (Plasmid No. 27437) and the AAV was packaged by University of Massachusetts Medical School (UMMS), with a titer of 1.4*10^13 genome copies/ml.

Stereotaxic surgery

Methods for stereotaxic surgery were described previously (*17, 19*). Stereotactic viral injections, microendoscope implants, and optic fiber implants were all performed in accordance with MIT's CAC guidelines. Mice were anaesthetized using 500 mg/kg avertin. Viruses were injected using a glass micropipette attached to a 10 ml Hamilton microsyringe through a microelectrode holder filled with mineral oil. A microsyringe pump and its controller were used to control the speed of the injection. The needle was slowly lowered to the target site and remained for 5 min after the injection. Three jewelry screws were placed on the skull surrounding the implant site of each hemisphere to provide additional stability. A layer of adhesive cement was applied followed by dental cement to secure the optical fiber implant. A cap made from the top part of an Eppendorf tube was used to protect the implant. The incision was closed with sutures. Mice were given 1.5 mg/kg metacam as analgesic and remained on a heating pad until fully recovered from anaesthesia. Mice were allowed to recover for 2 weeks before all subsequent experiments.

For behavioral experiments, bilateral viral delivery and optic fiber implants were aimed at coordinates relative to Bregma: vHPC injections were targeted to (AP: - 3.08 mm, ML: ± 3.75 mm, DV: - 3.70 mm). vCA1 injections were targeted to (AP: - 3.16 mm, ML: ± 3.10 mm, DV: - 4.70 mm). dCA1 injections were targeted to (AP: - 2.00 mm, ML: ± 1.50 mm, DV: - 1.50 mm). vHPC implants were placed at (AP: - 3.08 mm, ML: ± 3.75 mm, DV: - 3.45 mm). vCA1 implants were placed at (AP: - 3.16 mm, ML: ± 3.10 mm, DV: - 4.55 mm). dCA1 implants were placed at (AP: - 2.00 mm, ML: ± 1.50 mm, DV: - 1.30 mm). OB implants were placed at (AP: + 2.22 mm, ML: ± 0.60 mm, DV: - 3.55 mm). NAc implants were placed at (AP: + 1.34 mm, ML: ± 0.60 mm, DV: - 4.00 mm). BLA implants were placed at (AP: - 2.20 mm, ML: ± 3.50 mm, DV: - 4.70 mm). AAV8-CaMKIIa:eArchT3.0-EYFP volume was 200 nl for vHPC and 300 nl for dHPC of C57BL/6J mice. AAV9-hsyn:DIO-eArchT3.0-EYFP volume was 200 nl for vCA1 in Trpc4-Cre mice or 150 nl for dCA1 in Wfs1-Cre mice. AAV9-EF1α:DIO-hChR2(H134R)-EYFP or AAV9-EF1α:DIO-EYFP volume was 150 nl for vCA1. AAV9-TRE:ChR2-EYFP and AAV9-TRE:EYFP volumes were 200 nl for vCA1 and 100 nl for dCA1.

For CTB experiments, unilateral CTB-Alexa555 delivery into the right OB, NAc or BLA of wild-type/Trpc4-Cre mice were aimed at coordinates relative to Bregma: OB injections were targeted bilaterally to (AP: + 2.22 mm, ML: + 0.70 mm, DV: - 3.80 mm). NAc injections were targeted bilaterally to (AP: + 1.34 mm, ML: + 1.00 mm, DV: - 3.90 mm). BLA injections were targeted bilaterally to (AP: - 2.20 mm, ML: + 3.50 mm, DV: - 4.85 mm). 12 to 16 week-old mice were injected with 75 nl of CTB-Alexa555 (1% wt/vol) and perfused 7 days later.

For reactivation overlap experiments, bilateral viral delivery of AAV9-TRE:FT-Slow or AAV9-TRE:H2B-EGFP was aimed at coordinates relative to Bregma: vCA1 injections were targeted to (AP: - 3.16 mm, ML: ± 3.10 mm, DV: - 4.70 mm). AAV9-TRE:FT-Slow volume was 150 nl for vCA1 in c-fos:tTA without GFP transgenic mice. AAV9-TRE:H2B-EGFP volume was 300 nl for vCA1 in c-fos:tTA without GFP transgenic mice.

For histology, unilateral viral delivery into right dCA2 of Map2k15-Cre mice was aimed at coordinates relative to Bregma: dCA2 injections were targeted to (AP: - 1.60 mm, ML: + 1.60 mm, DV: - 1.70 mm). The 12 to 16 week-old Map2k15-Cre mice were injected with 200 nl of AAV9-hsyn:DIO-eArchT3.0-EYFP and perfused 2 weeks later.

For vCA1 Ca^{2+} imaging, unilateral AAV5-Syn:DIO-GCaMP6f injections into right vCA1 of Trpc4-Cre mice and right dCA1 of Wfs1-Cre mice were aimed at the following coordinates relative to Bregma: vCA1 injections were targeted to (AP: - 3.30 mm, ML: + 3.75 mm, DV: - 3.90 mm), dCA1 injections were targeted to (AP: - 2.00 mm, ML: + 1.40 mm, DV: - 1.55 mm). A microprism grin lens for vCA1 (outer diameter, 1.0 mm) was implanted at AP - 3.30 mm, ML + 4.40 mm (center of the lens) / + 3.90 mm (medial side of the lens), DV - 4.00 mm (medial side of the lens). A grin lens for dCA1 (outer diameter, 1.0 mm) was implanted as previously described (*20*).

Histology and immunohistochemistry

Methods for immunohistochemistry were previously described (*19*). Briefly, adult mice were transcardially perfused with 4% paraformaldehyde (PFA) in phosphate buffered saline (PBS). 50 μm brain sections were prepared using a vibratome, followed by

incubation in 0.3% Triton-X PBS with 5% normal goat serum for 1 hour at room temperature (RT). Primary antibodies were added to 5% NGS / 0.3% Triton-X in PBS solution and incubated overnight at 4°C. Primary antibodies: chicken anti-GFP (Invitrogen, A10262, 1:1000), mouse anti-tyrosine hydroxylase (TH; Millipore, MAB318, 1:1000), mouse anti-CaMKIIα (Abcam, ab22609, 1:200), mouse anti-GAD67 (Millipore, MAB5406, 1:1000), rabbit anti-CalbindinD-28K (Abcam, ab11426, 1:1000), mouse anti-RGS14 (NeuroMab, 75-170, 1:200), rabbit anti-wfs1 (Proteintech Group Inc, 11558-1-AP, 1:500), and rabbit anti-c-Fos (Santa Cruz, sc-52, 1:500). After rinsing with 1x PBS 3 times for 15 minutes each, tissue sections were incubated with Alexa Fluor-488, Alexa Fluor-546 or Alexa Fluor-633 conjugated secondary antibodies (Invitrogen, 1:500; for RGS14 staining, 1:200) for 3 hours at RT. Sections were then washed in 1x PBS 3 times for 15 minutes and mounted using VECTASHIELD medium on glass slides. Excluding FT-slow sections, all samples were stained by DAPI (1 μg/ml) for 15 min. IHC was not performed on FT-Slow sections. Fluorescence images were taken by confocal microscopy using 10X, 20X, and 40X objectives, as well as by fluorescence microscopy using 10X, 20X objectives. Z-projected confocal images were generated by Zen Black software. *In situ* hybridization and X-gal staining were performed as previously described (*33*).

Social discrimination test

All behavioral experiments were performed using 12 to 20 weeks old mice, during the facility light cycle. Behavioral subjects were individually habituated to the investigator by handling for several minutes on each of two separate days (Day-1 and Day-2). Handling took place in the holding room where the mice were housed. Habituation to the social discrimination chamber was performed for 10 min on each of two separate days (Day-2 and Day-3). Social discrimination chamber is a rounded rectangle composed of 30 x 30 cm square (center part) and half circle with a radius of 15 cm on both ends (30 cm height). The subject-mice were placed in a pencil holder, with dimensions of: circle with a radius of 7.5 cm and 15 cm height. On the evening of Day-3, a subject-mouse (B6129SF2, 6 to 8 weeks of age) was put into the home cage of the test-mice for

familiarization (total duration of 72 h, from Day-3 to Day-6). Social discrimination tests were performed on Day-6 and Day-7. Half of the test-mice were assessed under OFF-laser conditions on Day-6 and ON-laser conditions on Day-7, while the other half received a counterbalanced protocol. On the experimental day, subject-mice (i.e., familiar mice) were removed for 30 min before behavioral recording (interstimulus interval, ISI = 30 min). During the last 5 min of the ISI, a test-mouse was placed into the social chamber and allowed to explore. Behavioral recording and tracking were performed for 10 min by Ethovision XT software, using an infra-red (IR) sensitive GIG-E camera with two IR illuminators under dark conditions. For half of the behavioral assays, a novel subject-mouse was placed in the left holder and familiar subject-mouse in the right, while the remaining assays were run in a counterbalanced manner. 3-5 seconds is needed for detection of the test-mouse behavior by Ethovision software and 9 min recording data following detection was used. Total duration during which the nose-position of test-mice was in each sniffing area, a circle centered on the pencil holder with a radius of 11.25 cm (1.5 times the holders' radius), was measured. Behavior-triggered inhibition and activation experiments (Fig. 2, K to N and Fig. 4J) were performed with minor modifications of the previously described methods (*34*). The target zone for light stimulation was determined by a circle centered on the pencil holder with a radius of 15 cm (2 times the holders' radius). Only during the ON phases, when the nose-position of test-mice entered the target zone, a TTL signal triggered the stimulus generator. The signal from the stimulus generator activated the lasers. A 473 nm blue laser (473nm-600mW) and 561 nm green laser (561-300mW) were used for ChR2-activation and eArchT-inhibition, respectively. 15 ms and 20 Hz blue light pulses were used for ChR2 experiments, whereas constant green light was used for eArchT experiments. For 2h inhibition experiments (fig. S8), 10 epochs of 11 min green laser-ON followed by 1 min laser-OFF was used. Laser output was tested at the beginning of every experiment to ensure that at least 10 mW (blue laser) and 15 mW (green laser) of power were delivered to the ends of the optic fiber patch-cords. The discrimination score was calculated by the following equation (Duration $_{\text{novel}}$, total duration in the sniffing area of a novel mouse; Duration $_{\text{familiar}}$, total duration in the sniffing area of a familiar mouse).

$$\text{Discrimination score} = \frac{Duration_{novel} - Duration_{familiar}}{Duration_{novel} + Duration_{familiar}}$$

Resident - intruder test

Mouse handling, habituation, familiarization, and separation procedures were identical to SDT, described above and illustrated in fig. S1. After 30 min ISI, a familiar or novel subject-mouse was placed into the home cage of the test-mouse, and behavior was recorded for 3 min. Sniffing duration was manually measured using Observer software by a well-trained researcher under blind experimental conditions.

Novel/familiar object test

Mouse handling and habituation procedures are identical to SDT (Day-1 to 3). In the social discrimination chamber (without pencil holders), test-mice were allowed to explore two identical objects (A, batteries) for 6 min on Day-4, Day-5, and Day-6. On Day-7, test-mice interacted with familiar object-A and a novel object-B (an Erlenmeyer flask). Half of the test-mice were assessed under OFF-laser conditions while the remaining mice under ON-laser conditions. For half of the behavioral assays, familiar object-A was placed on the left and novel object-B on the right. Object locations were switched for the remaining assays. Behavior recording conditions and laser conditions were identical to SDT.

Novel/familiar context test

Mouse handling procedure is identical to SDT (Day-1 and Day-2). Social discrimination chamber without pencil holders was used as context-A, whereas a black rectangular arena (30 cm x 50 cm x 30 cm) with mesh paper flooring was used as context-B. Test-mice were allowed to explore context-A for 10 min on Day-3, Day-4, and Day-5. On Day-6 and Day-7, half of the test-mice explored context-A (A-A-A-A group), whereas the others explored context-B (A-A-A-B group). Half of the test-mice were assessed under

OFF-laser conditions on Day-6 and ON-laser conditions on Day-7, while the other half received a counterbalanced protocol. Behavior recording conditions and laser conditions were identical to SDT.

Optogenetic reactivation of social memory

Mouse handling and habituation procedure were identical to SDT. All c-fos:tTA test-mice were raised on a 40 mg/kg doxycycline (Dox) diet until the evening of Day-3 (ON-Dox). Prior to labeling, test-mice were given food without Dox for 48 hours (Day-4 and Day-5, OFF-Dox). On the evening of Day-5, a subject-mouse (B6129SF2, 6-8 weeks of age) was put into the home cage of the test-mouse for ChR2 labeling during a 2 h social interaction. Immediately after social interaction and removal of the subject-mouse from the home cage, test-mice were returned to 40 mg/kg Dox (ON-Dox). On Day-6 (24 hours after social interaction), SDT was performed for 10 min during blue laser stimulation (12 mW, 15 ms light pulses, 20 Hz; ON-laser group) or no stimulation (OFF-laser group) conditions.

Analysis of social memory engram cells (memory inception)

Mouse handling, habituation, and ChR2-labeling procedures during social interaction were identical to those for optogenetic reactivation of social memory. Additionally, habituation to the fear-conditioning chamber (30 x 35 x 32 cm) was also performed for 10 min on Day-2 and Day-3. On Day-6, pre-test was performed including an identical behavioral recording procedure as SDT. On Day-7 and Day-8, fear or reward unconditioned stimuli was applied during blue laser stimulation (12 mW, 15 ms light pulses, 20 Hz) twice per day for two days. For fear-conditioning, test-mice were placed in the fear-conditioning chamber for 500 s and foot shocks (0.75 mA, 2 s duration) were administered at 198 s, 278 s, 358 s and 438 s time points. For reward conditioning, test-mice were placed in the fear conditioning chamber 20 min after cocaine injection (20 mg/kg, intraperitoneal injection) and were applied with laser stimulation for 15 min. On Day-9, shock-test or cocaine-test was performed using a similar protocol as the pre-test.

To compare durations in mouse-A area between before and after memory inception, the durations in mouse-A area and that in mouse-B area were normalized by sum of the durations in mouse-A area and that in mouse-B area, which is shown as the proportion of total duration (%) in Fig. 4L, M, O, and P.

Ca^{2+} imaging by the microendoscope

Calcium imaging was performed on male Trpc4-Cre (for vCA1) or Wfs1-Cre (for dCA1) mice in the animal facility during the light cycle. Two weeks following AAV5-hsyn:DIO-GCaMP6f injection, a microprism lens was implanted targeting vCA1/dCA1. Two weeks later, a baseplate for the miniaturized microscope camera (*20*) was attached above the implanted lens. Following surgery, test-mice were habituated to experimenters as well as the social discrimination chamber containing pencil holders for subject-mice in the experimental room for 15 min each on 3 separate days. Behavior and Ca^{2+} signals of test-mice were recorded in the social discrimination chamber for three, 5 min recording sessions (total 15 min). During the first 5 min, mouse-A was on the left while mouse-B on the right. During the second 5 min, the position was switched to counterbalance the experiments. During the last 5 min, there were only pencil holders without subject-mice. The social discrimination chamber and pencil holders were cleaned with water between sessions. These sessions were performed three times every 3 days (Before, After-1 (30 min), and After-2 (30min)) or two times with a 2 h familiarization period. Test-mice were familiarized with mouse-A in their home cages between recordings (as described in Fig. 3F) and subject-mice (i.e., familiar mice) were removed for 30 min before recording. For comparison between 30 min and 24 h after separation (Fig. 3, N to P), Ca^{2+} signals were recorded 24 h after the After-1 (30 min) recording (After-1 (24 h)). Recording conditions were identical to SDT except the IR-illuminator was not used since microendoscopes detect IR light. The IR-sensitive camera detected blue light from the microendoscope and tracking of the light was performed by Ethovision software. Calcium events were captured at 20 Hz on an Inscopix miniature microscope. The recorded calcium-imaging movie was processed as described in (*35*) with minor modifications. Motion correction of the movie was performed using Inscopix Mosaic software (correction type, translation,

rotation, and scaling; reference region by subtracting spatial mean [r = 20 pixels], inverting, and applying spatial mean [r = 5 pixels]). Motion corrected movies were processed by ImageJ (dividing each image, pixel by pixel, by a low-pass [r = 20 pixels] filter) to fix the base line, and subsequently the $\Delta F/F$ signal was calculated by Mosaic. After acquiring a stacked image from a 15 min recording by maximum intensity projection, cell locations were carefully selected manually, as small regions of interest (ROIs) at the center of the cell bodies using ImageJ. Approximately 70 cells were selected per animal, and their $\Delta F/F$ signals were isolated. Ca^{2+} events were detected by applying a threshold (> 3% from the $\Delta F/F$ signal at the local maxima). Statistical definition for identifying mouse-A or mouse-B neurons is described in fig. S12. Event probability was obtained by dividing the number of Ca^{2+} events in mouse-A area or mouse-B area of target neurons by total duration in mouse-A or mouse-B areas, respectively.

c-Fos counting

After acquiring confocal images (single optical planes of 10 μm), H2B-EGFP expressing cells were identified using ImageJ. Noise was removed by applying a threshold "remove outlier" (radius, 2.0; threshold, 50; dark outlier). vCA1 was manually identified and regions outside vCA1 was removed by a "clear outside" command. ROIs were identified by "analyzed particles" (size, 150 to 1000; circularity, 0.3 to 1.0) and fluorescence intensities of c-Fos images in each ROI were measured as absolute fluorescence magnitude. To calculate a random shuffling score for each sample (random fluorescence score), average fluorescence intensities of c-Fos images from a different mouse was used. The absolute fluorescence scores minus the random fluorescence score provided the normalized c-Fos fluorescence intensity.

FT-Slow analysis

Adult mice were transcardially perfused with 4% PFA for 2 h at room temperature. Immediately following perfusion, 50 μm brain sections were collected using a vibratome.

Fluorescence images were acquired by confocal microscopy. Methods for identification of FT-Slow red form expressing cells using ImageJ and normalization processes are identical to those used for c-fos counting. An ROI, whose mean of FT-Slow red form signal was over 80, was identified as a FT-Slow red form positive cell. FT-Slow blue form signal in FT-Slow red form positive cells were calculated and histograms were plotted (fig. S13, E and F). Among them, an ROI, whose FT-Slow blue form signal was over 80, was identified as a FT-Slow blue form positive cell and the percentage of reactivated cells was calculated (Fig. 4E).

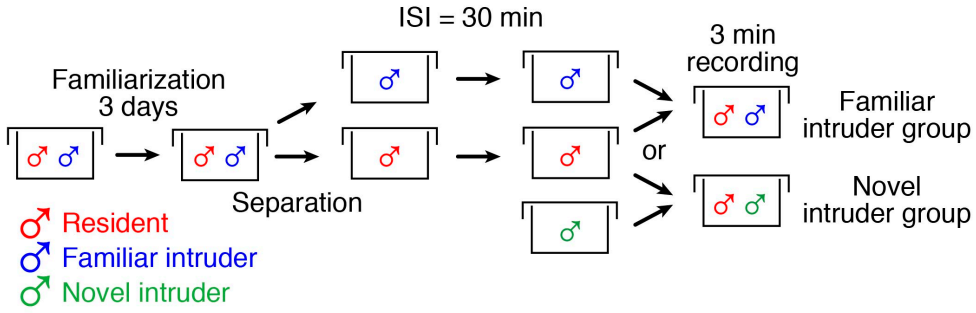

Fig. S1. Behavioral schedule for the resident-intruder test (RIT).

Experimental procedure. A resident male mouse (red) was familiarized with an intruder male mouse (blue, familiar intruder) for 3 consecutive days. On the behavioral test day, the familiar intruder was removed from the home cage for 30 min (interstimulus interval, ISI). After this interval, the familiar male mouse-A or a novel male mouse-B (green, novel intruder) was introduced into the resident's home cage. Behavior was recorded for 3 min and the total sniffing duration by resident to intruder was manually scored.

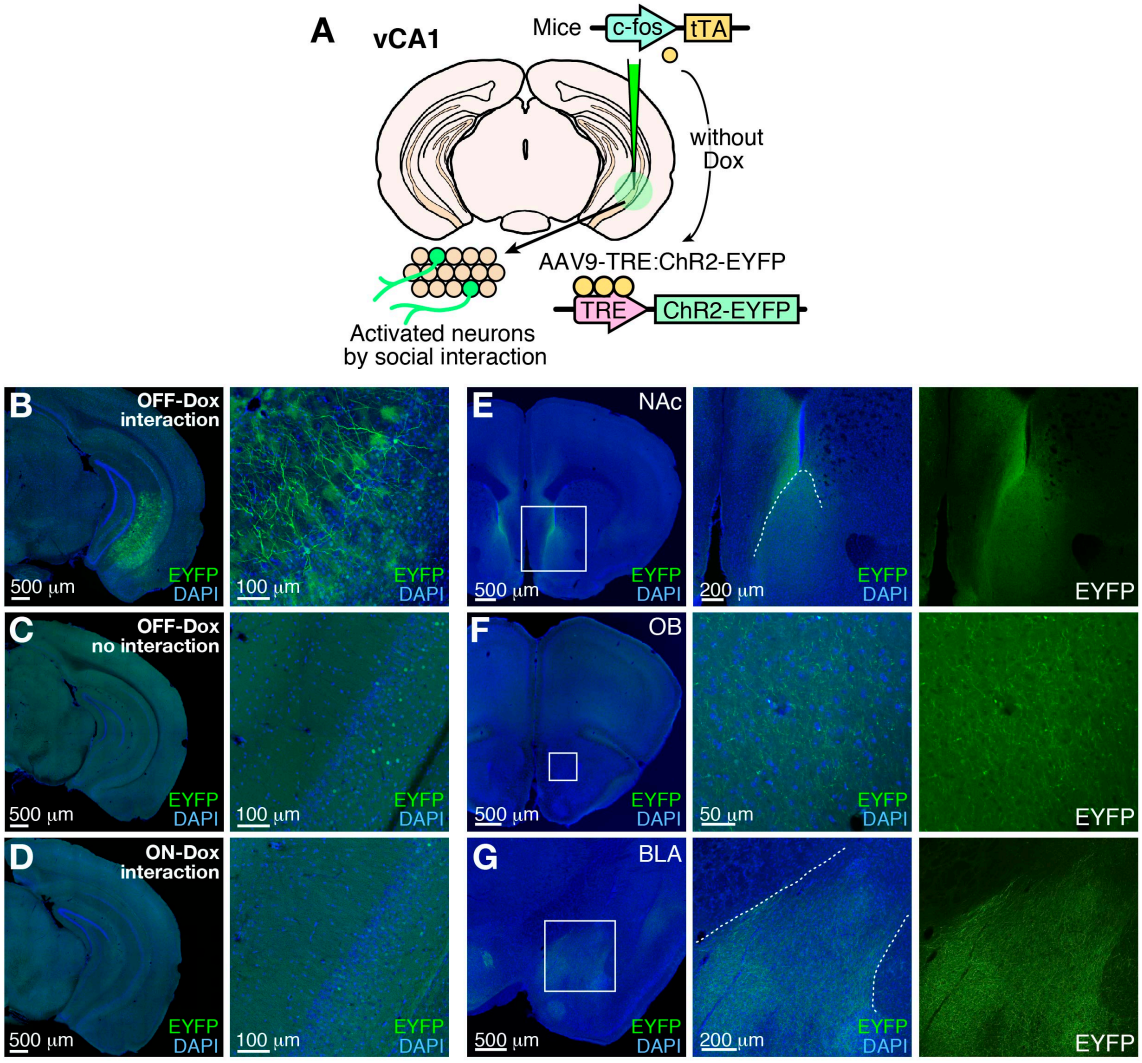

Fig. S2. Examining the brain-wide projection pattern of social interaction-specific vCA1 neurons.

(**A**) Activity-dependent labeling using c-fos:tTA mice combined with AAV9-TRE:ChR2-EYFP. Ventral CA1 (vCA1) neurons activated by social interaction were tagged with ChR2-EYFP. (**B** to **D**) Social interaction-induced ChR2-EYFP expression; (**B**) interaction during OFF-Dox period, (**C**) no interaction during OFF-Dox period, and (**D**) interaction during ON-Dox period. (**E** to **G**) Projections of activated neurons were observed in NAc (**E**), OB (**F**), and BLA (**G**). Coronal sections containing vCA1 were stained with anti-GFP (green) and DAPI (blue). White boxed areas in left panels are magnified in middle and right images.

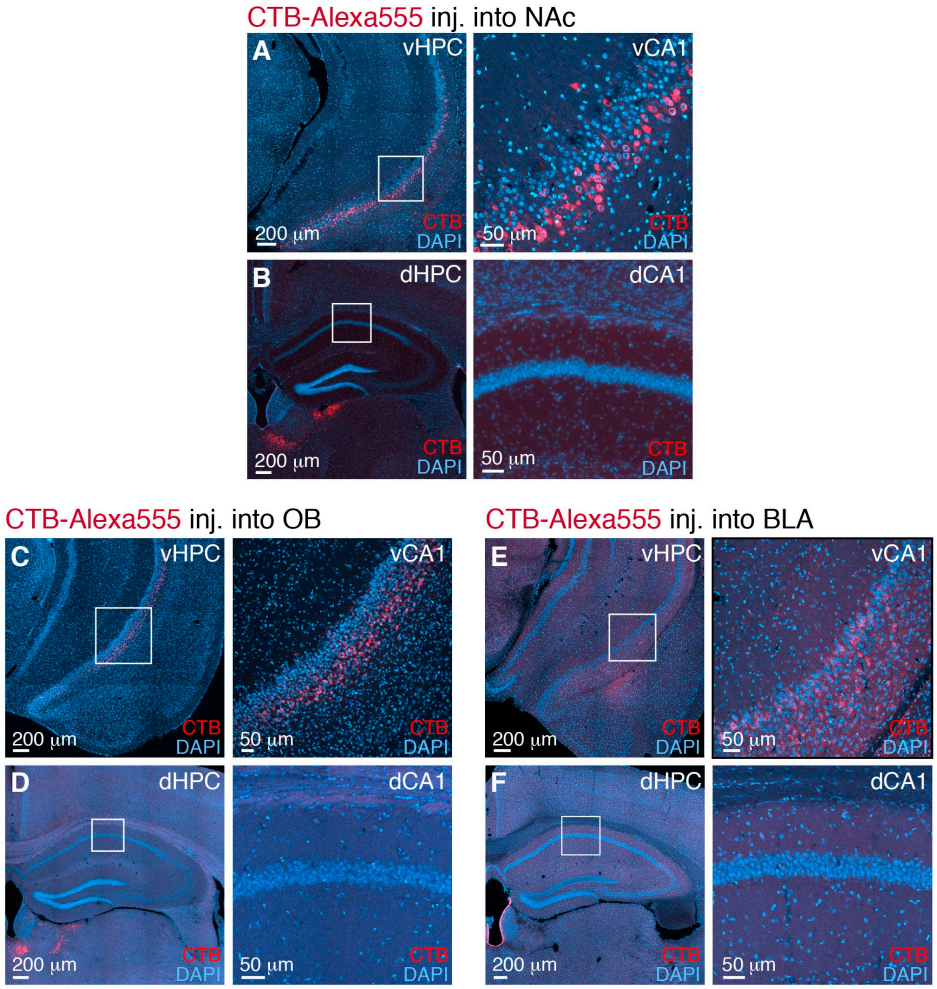

Fig. S3. Retrograde tracer injection into NAc, OB, and BLA.

Coronal sections of CTB-Alexa555 (red) injected mice. Sections were stained with DAPI (blue). CTB-Alexa555 injection into NAc (**A**, **B**), OB (**C**, **D**), or BLA (**E**, **F**). White boxed areas of left panels are magnified in right panels. vHPC sections (**A**, **C**, **E**) and dHPC sections (**B**, **D**, **F**).

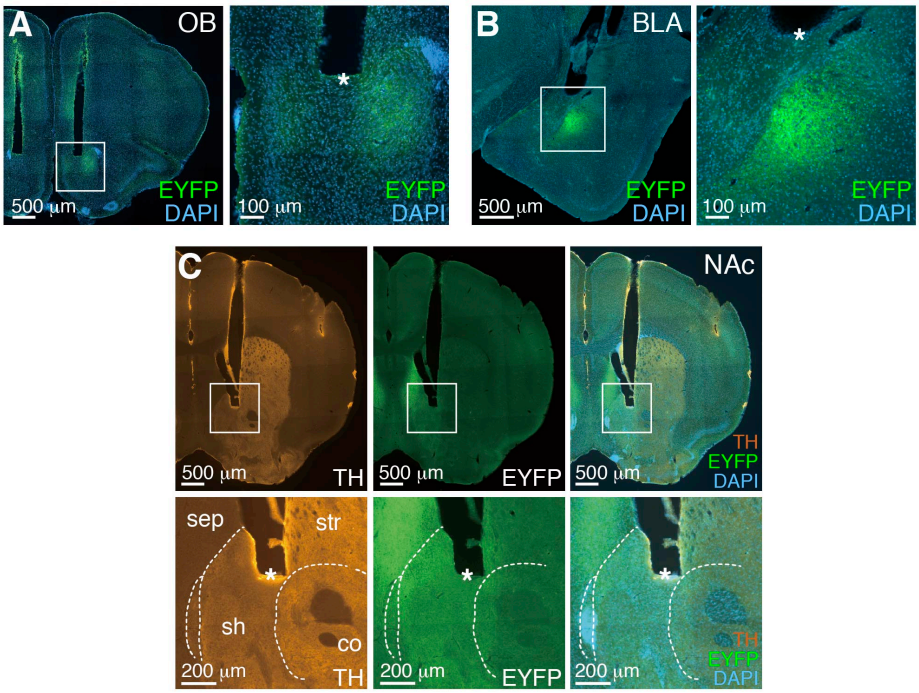

Fig. S4. Histological verification of AAV expression and optic fiber position.

Coronal sections of wild-type mice bilaterally injected with AAV8-CaMKII:eArchT-EYFP into vHPC and implanted with optic fibers targeting to OB (**A**), BLA (**B**) and NAc (**C**). Sections were stained; (**A**, **B**) with anti-GFP (green) and DAPI (blue); (**C**) with anti-GFP, anti-TH (orange), and DAPI (blue). Asterisk indicates optic fiber tip. White boxed areas are magnified. NAc shell (sh); NAc core (co); Septum (sep); Striatum (str).

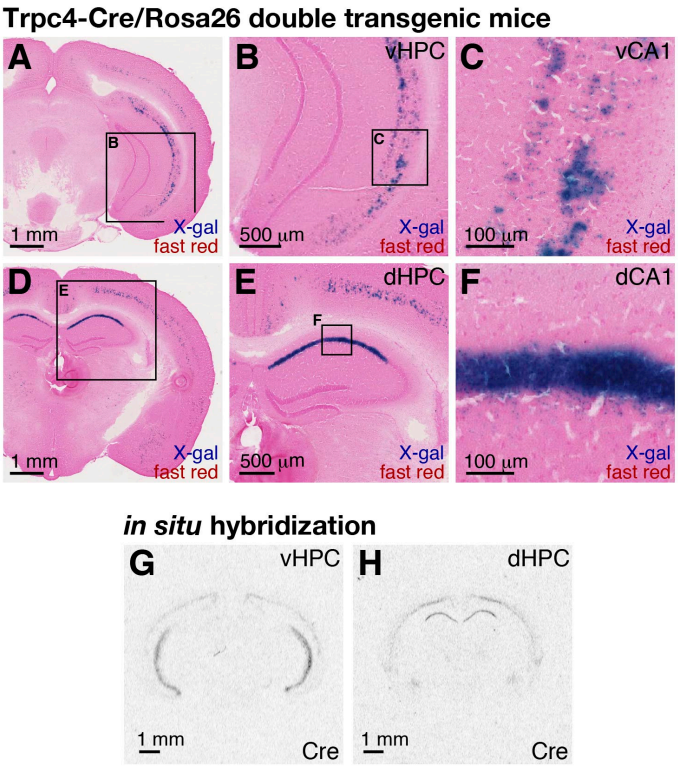

Fig. S5. Verification of Cre-loxP recombination in Trpc4-Cre/Rosa26 double transgenic mice.

(**A** to **F**) Coronal vHPC and dHPC sections of a Trpc4-Cre/Rosa26 double transgenic mouse. Sections were stained with X-gal (blue) and nuclear fast red (red). Cre-loxP recombination was restricted to vCA1 (**B** and **C**) and dCA1 (**E** and **F**) in the hippocampus. Black boxed areas are magnified. (**G**, **H**) Coronal vHPC and dHPC sections of a Trpc4-Cre mouse. Cre mRNA expression was localized to vCA1 (**G**) and dCA1 (**H**) in the hippocampus, which was revealed by *in situ* hybridization.

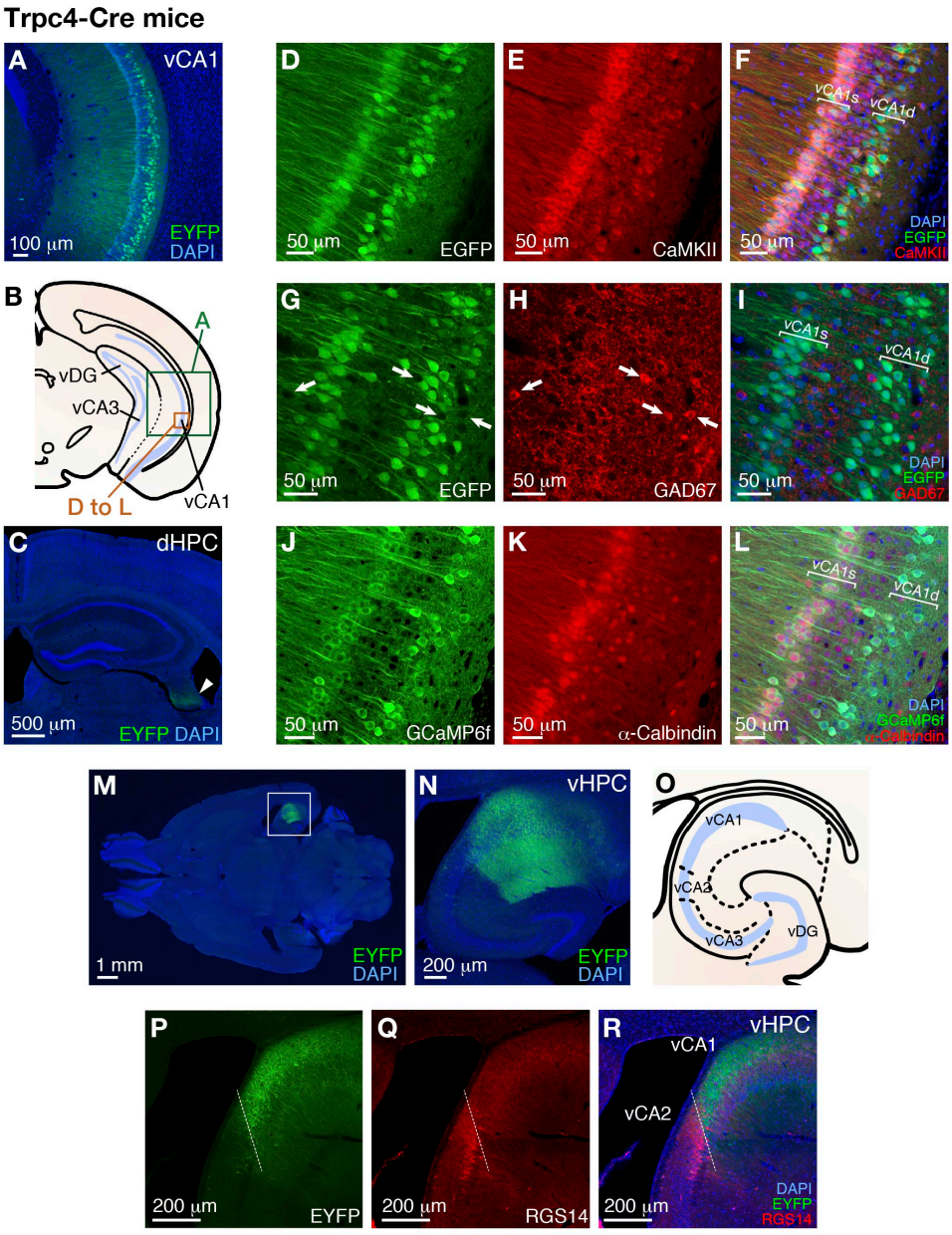

Fig. S6. Characterization of Cre expression in Trpc4-Cre mice.

(**A** to **L**) Coronal sections of Trpc4-Cre mice injected with AAV9-hsyn:DIO-eArchT-EYFP (**A**, **C**), AAVrh8-syn:DIO-EGFP (**D** to **I**), and AAV5-hsyn:DIO-GCaMP6f (**J** to **L**) into vCA1. Sections were stained with; (**A**, **C**) anti-GFP (green) and DAPI (blue); (**D** to **F**) anti-GFP (green, **D**), anti-CaMKII (red, **E**), and DAPI (blue); (**G** to **I**) anti-GFP (green, **G**), anti-GAD67 (red, **H**), and DAPI (blue); (**J** to **L**) anti-GFP (green, for GCaMP6f, **J**), anti-α-Calbindin (red, **K**), and DAPI (blue). (**F**, **I**, **L**) Merged images. (**A**, **D** to **L**) vCA1 sections and (**C**) dHPC section (white arrow head, axons from vCA1 neurons in the fimbria of hippocampus). (**M** to **R**) Horizontal vHPC sections of Trpc4-Cre mice injected with AAV9-hsyn:DIO-eArchT-EYFP. Sections were stained with (**M**,

N) anti-GFP (green) and DAPI (blue); (**P** to **R**) anti-GFP (green, **P**), anti-RGS14 (red, **Q**), and DAPI (blue). (**R**) Merged images. (**B**, **O**) Schematic drawings of coronal and horizontal vHPC sections, respectively. White boxed areas (**M**) are magnified in (**N**).

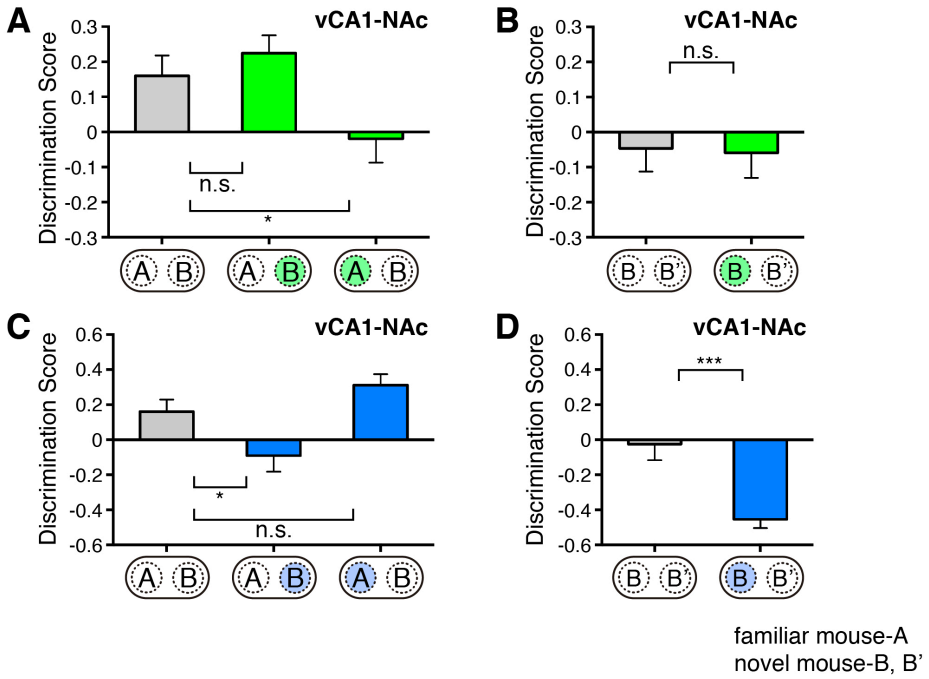

Fig. S7 Comparison of discrimination scores.
mouse-A is a familiar mouse, while mouse-B and -B' are novel mice. Comparison of Discrimination Scores between laser-OFF and laser-ON groups of Fig. 2K (**A**), 2L (**B**), 2M (**C**), and 2N (**D**). The Discrimination Score is defined as (duration $_{novel}$ - duration $_{familiar}$)/(duration $_{novel}$ + duration $_{familiar}$). Significance for multiple comparisons: **A**, **C**, Dunnett's test; **B**, **D**, t-test; *$p < 0.05$; ***$p < 0.001$, n.s., not significant. Data presented as mean ± S.E.M.

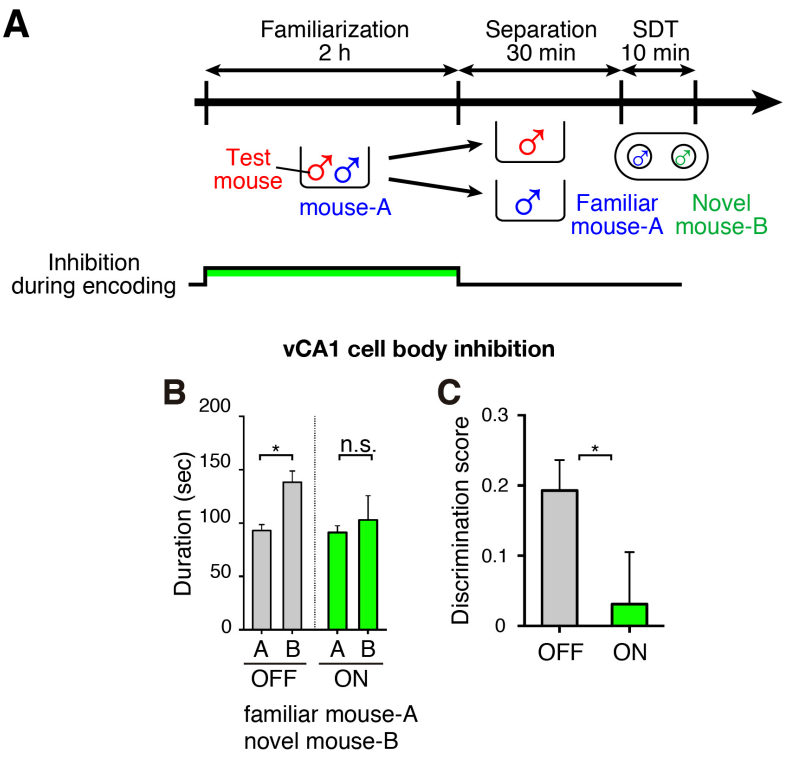

Fig. S8 Optogenetic inhibition of vCA1 neurons during social memory encoding. mouse-A is a familiar mouse, while mouse-B is a novel mouse. (**A**) Behavioral schedule. During familiarization period (2 h), light was delivered to inhibit vCA1 neurons (10 times of 11 min laser-ON followed by 1 min laser-OFF). SDT was performed followed by 30 min separation period. (**B**) Total duration in sniffing area during SDT observed in Trpc4-Cre mice bilaterally injected with AAV9-hsyn:DIO-eArchT-EYFP into vCA1 and implanted with optic fibers targeting vCA1. (**C**) Discrimination scores. Significance for multiple comparisons: paired t-test (**B**) and t-test (**C**), *p < 0.05; n.s., not significant. Data presented as mean ± S.E.M.

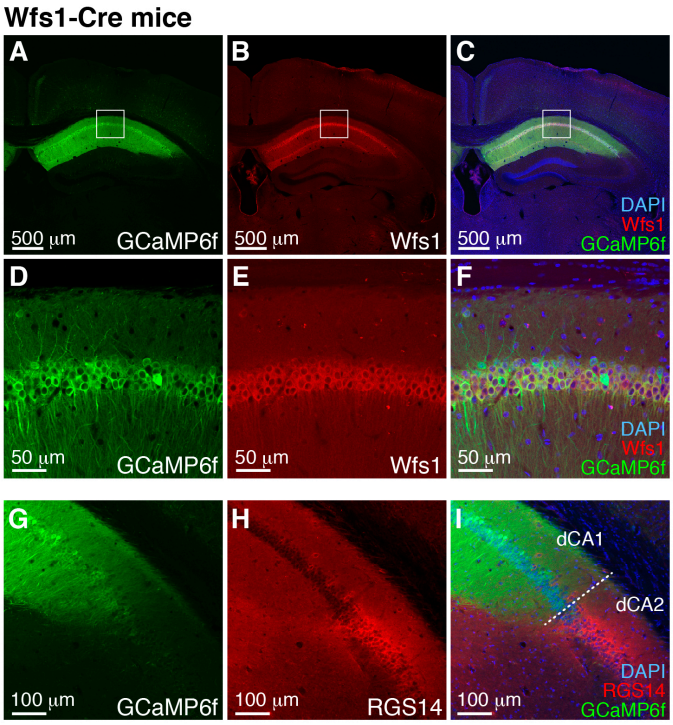

Fig. S9. Characterization of Cre expression in Wfs1-Cre mice.

Coronal dHPC sections of a Wfs1-Cre mouse injected with AAV5-hsyn:DIO-GCaMP6f into dCA1. (**A** to **F**) Sections were stained with anti-GFP (green, for GCaMP6f), anti-wfs1 (red), and DAPI (blue). GCaMP6f (**A**, **D**), wfs1 (**B**, **E**), and merged images (**C**, **F**). White boxed areas (**A** to **C**) are magnified in (**D** to **F**). (**G** to **I**) Sections were stained with anti-GFP (green), anti-RGS14 (red), and DAPI (blue). GCaMP6f (**G**), RGS14 (**H**), and merged image (**I**).

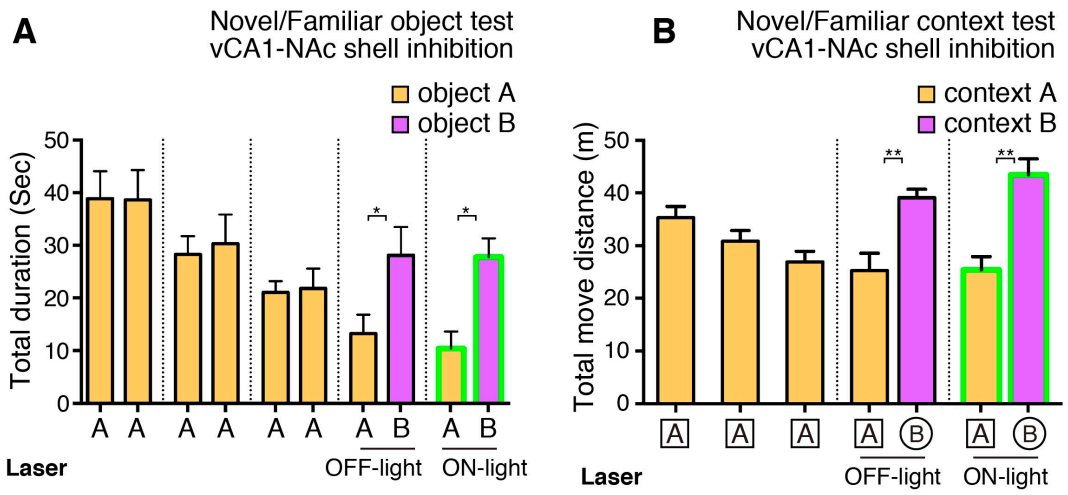

Fig. S10. Novelty detection paradigm during vCA1-NAc shell inhibition.

Trpc4-Cre mice received bilateral AAV9-hsyn:DIO-eArchT-EYFP injections into vCA1 followed by optic fibers targeting NAc shell. (**A**) Novel/familiar object test. Total sniffing duration was measured during a 6 min recording session with object A (yellow bar) or B (purple bar). Data with green borders, green laser-ON (with light stimulation, n = 9); data with black borders, laser-OFF (without light stimulation, n = 9). (**B**) Novel/familiar context test. Total distance traveled during a 10 min recording in context A (yellow bar) or B (purple bar). Data with green borders, green laser-ON (with light stimulation); data with black borders, laser-OFF (without light stimulation). A-A-A-A or A-A-A-B groups, n = 5 each. Significance for multiple comparisons: paired t-test (**A**) and t-test (**B**); *p < 0.05, **p < 0.01. Data are presented as mean ± S.E.M.

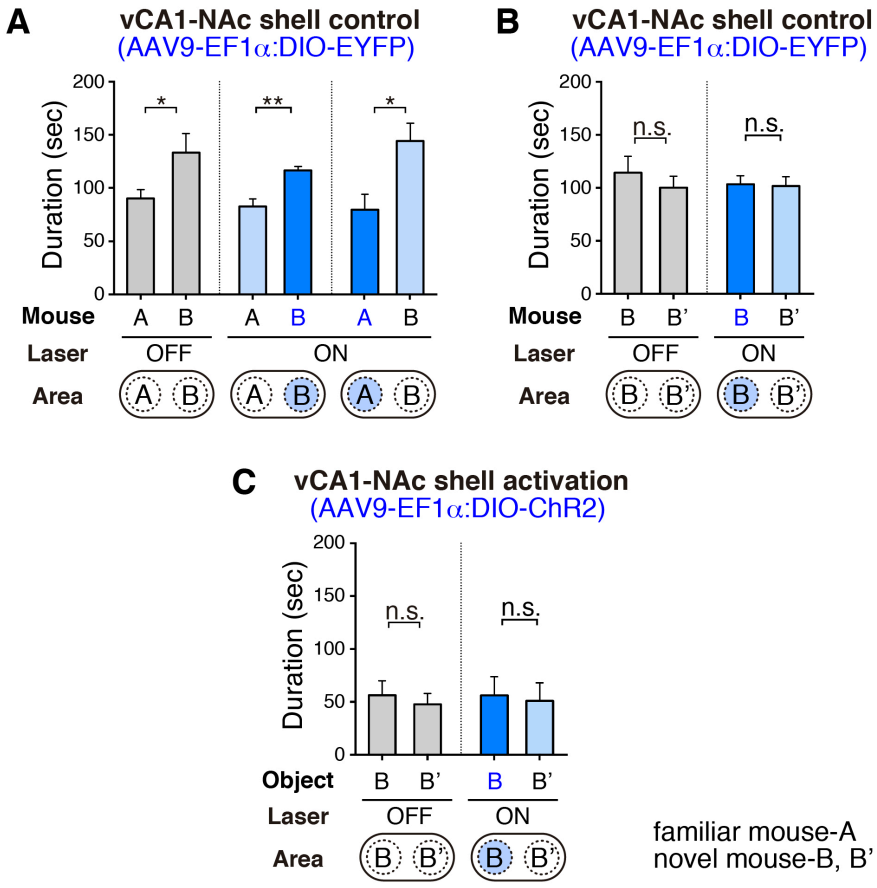

Fig. S11. Control behavioral tests for vCA1-NAc shell activation experiments.
mouse-A is a familiar mouse, while mouse-B and -B' are novel mice. (**A**, **B**) Total sniffing duration in SDT. Trpc4-Cre mice received bilateral AAV9-EF1α:DIO-ChR2-EYFP injections into vCA1 followed by optic fibers targeting NAc shell (n = 7). (**C**) Total sniffing duration in SDT. Trpc4-Cre mice received bilateral AAV9-EF1α:DIO-ChR2-EYFP injections into vCA1 followed by optic fibers targeting NAc shell (n = 7). For this control experiment, novel objects were used instead of novel mice. Bottom of each graph, target area for laser stimulation is indicated. Blue bars, blue laser-ON session; Grey bars, laser-OFF session. Significance for multiple comparisons: paired t-test; *$p < 0.05$, **$p < 0.01$, n.s., not significant. Data are presented as mean ± S.E.M.

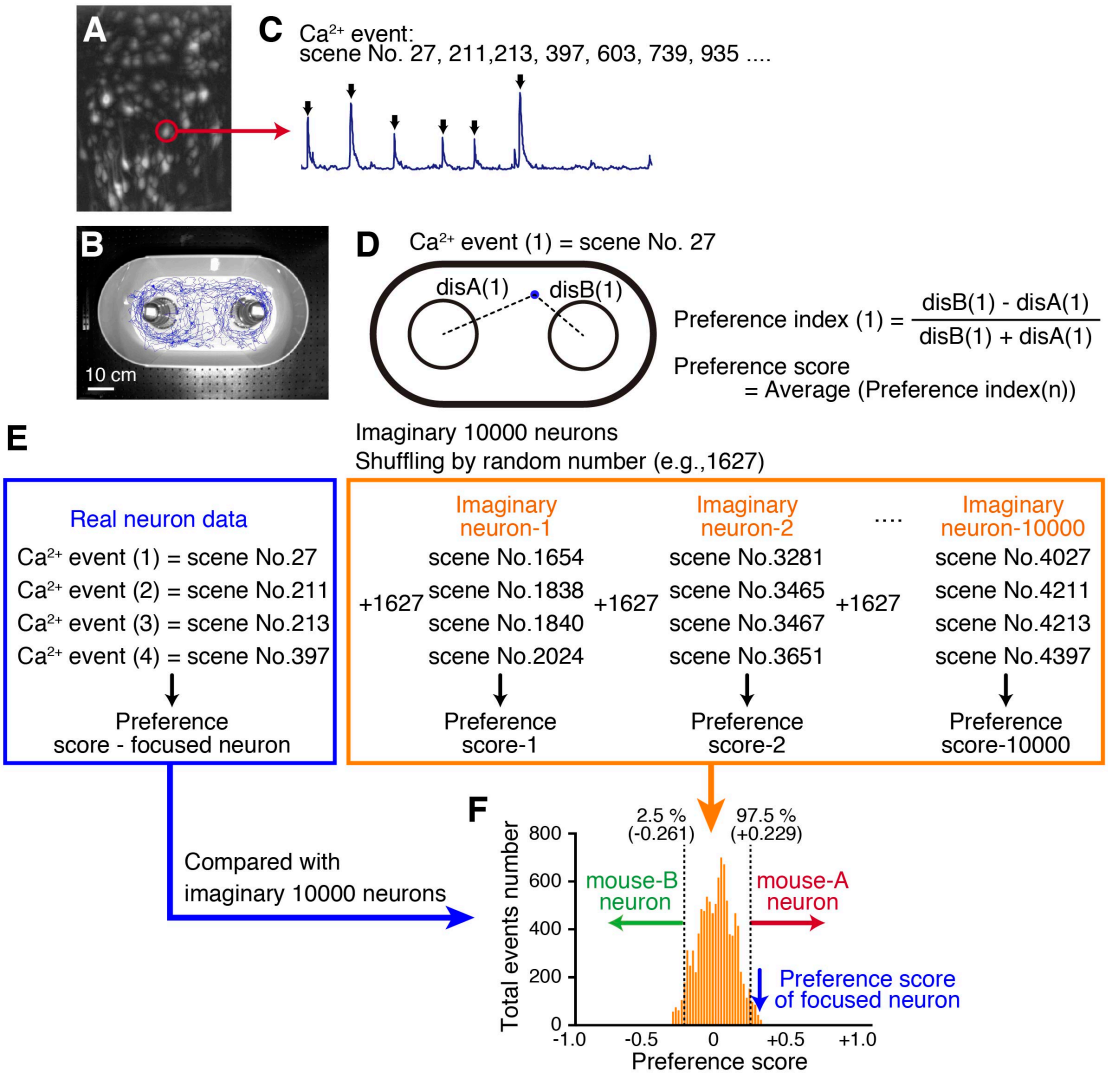

Fig. S12. Statistical definition for identifying mouse-A or mouse-B neurons in vCA1.

(**A**) Stacked vCA1 image acquired during a 15 min microendoscope recording session using Trpc4-Cre mice injected with AAV5-hsyn:DIO-GCaMP6f. Red circle indicates a neuron selected for Ca^{2+} event detection. (**B**) Social discrimination chamber containing representative behavioral tracking data for the test-mouse (blue line). (**C**) Representative Ca^{2+} activity. Black arrows show individual Ca^{2+} events. (**D**) Calculation of preference index for each Ca^{2+} event as well as preference score for individual neurons. (**E**) 10,000 imaginary neurons generated by shuffling. Preference score of the selected neuron (blue box) and preference scores of the 10,000 imaginary neurons (orange box). (**F**) Histogram of the 10,000 imaginary neuron data. Dashed lines representing 97.5% and 2.5% were calculated from the histogram in order to identify mouse-A or mouse-B neurons, respectively. The selected neuron whose preference score was over the 97.5% dashed line was identified as a mouse-A neuron.

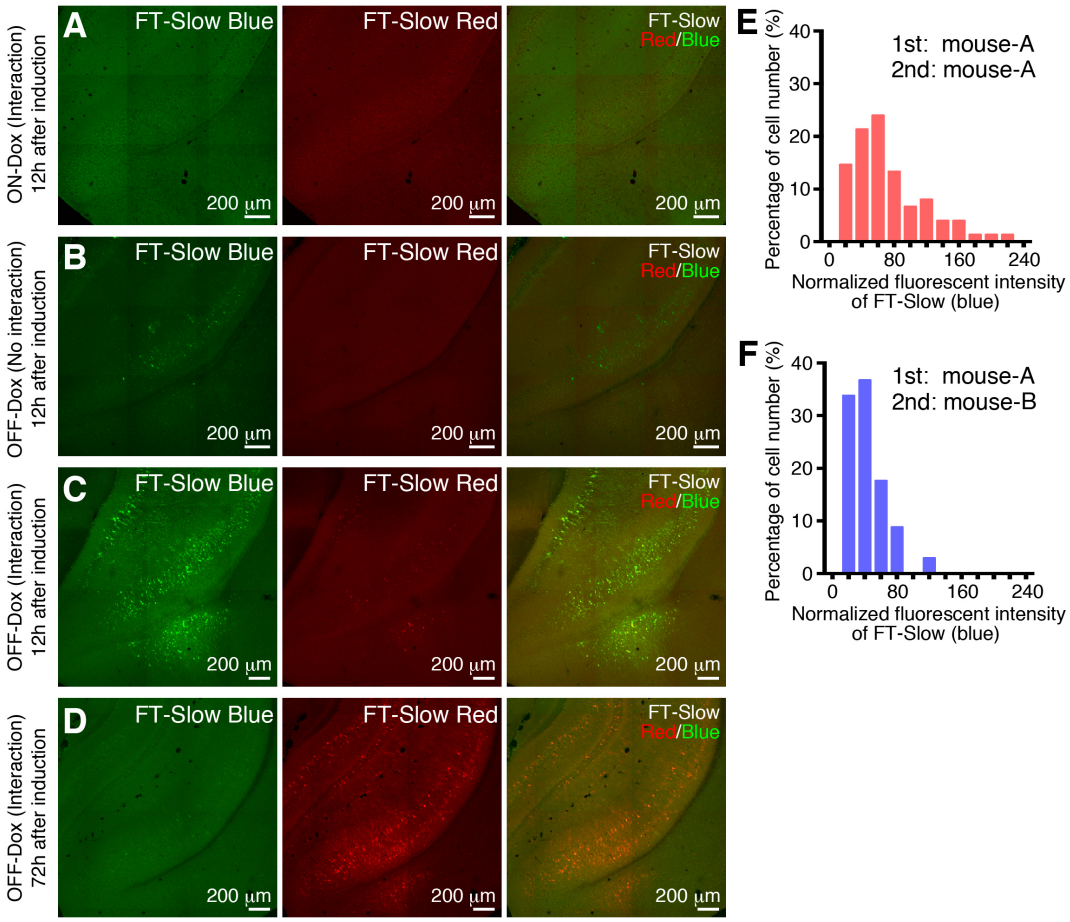

Fig. S13. FT-Slow expression in vCA1 neurons activated by social interaction.

(**A** to **D**) Coronal vCA1 sections of AAV9-TRE:FT-Slow injected c-fos:tTA mice. (**A**) Social interaction during ON-Dox period. (**B**) No interaction during OFF-Dox period. (**C**-**D**) Social interaction during OFF-Dox period (same as Fig. 4B). (**A** to **C**) 12 h after induction; (**D**) 72 h after induction. Left panels, FT-Slow blue image; Middle panels, FT-Slow red image; Right panels, merged image. (**E**, **F**) Histograms of normalized fluorescence intensity of FT-Slow (blue form) among FT-Slow (red form) positive cells. Regions of interest (ROIs) were defined by the red channel and mean of each ROI were calculated in the blue channel. (**E**) Same mice interaction group (A/A); (**F**) Different mice interaction group (A/B), n = 3 mice per group.

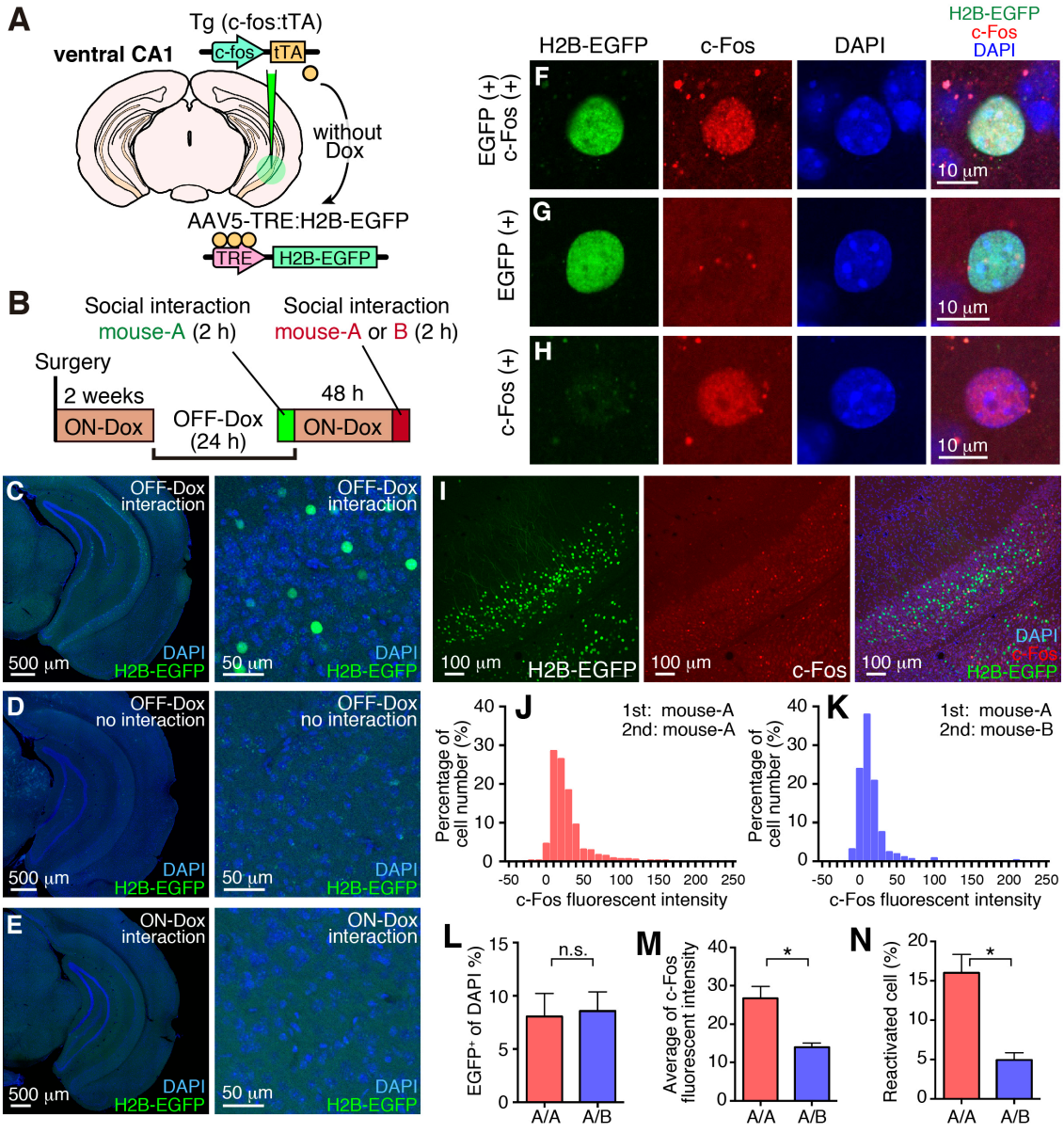

Fig. S14. Reactivation of vCA1 neuronal ensembles following interaction with familiar mice.

(**A**) c-fos:tTA without GFP mice injected with AAV5-TRE:H2B-EGFP into vCA1. (**B**) Experimental procedure. 24 h after Dox removal, test-mice interacted with mouse-A for labeling activated neurons with histone H2B-EGFP (nuclear localized EGFP). 48 h later, test-mice interacted with mouse-A or a different mouse (mouse-B). (**C** to **E**) Social interaction induced H2B-EGFP expression in vCA1. Coronal vHPC sections were stained with anti-GFP (green) and DAPI (blue). (**C**) Interaction during OFF-Dox. (**D**) No interaction during OFF-Dox. (**E**) Interaction during ON-Dox. (**F** to **I**) Coronal sections were stained with anti-GFP (green), anti-c-Fos (red), and DAPI (blue). Representative

neurons expressing EGFP and c-Fos (**F**), EGFP alone (**G**), and c-Fos alone (**H**). (**I**) Representative vCA1 coronal sections of a test-mouse. (**J** to **N**) Quantification of H2B-EGFP and c-Fos overlap. H2B-EGFP expressing neurons were automatically identified by ImageJ software and subsequently c-Fos fluorescence intensities were measured. Histogram of normalized c-Fos intensity of same mice interaction group (**J**) and different mice interaction group (**K**)**,** n = 3 mice per group. (**L**) H2B-EGFP cell counts from vCA1 sections of the same mice interaction group (A/A) and different mice interaction group (A/B) (n = 3 mice per group). (**M**) Average intensity of the same mice interaction group (A/A) and different mice interaction group (A/B) (n = 3 mice per group). (**N**) Percentage of reactivated cells (normalized c-Fos fluorescence intensity > 40) (n = 3 mice per group). Significance for multiple comparisons: t-test, *$p < 0.05$; n.s., not significant. Data are presented as mean ± S.E.M.

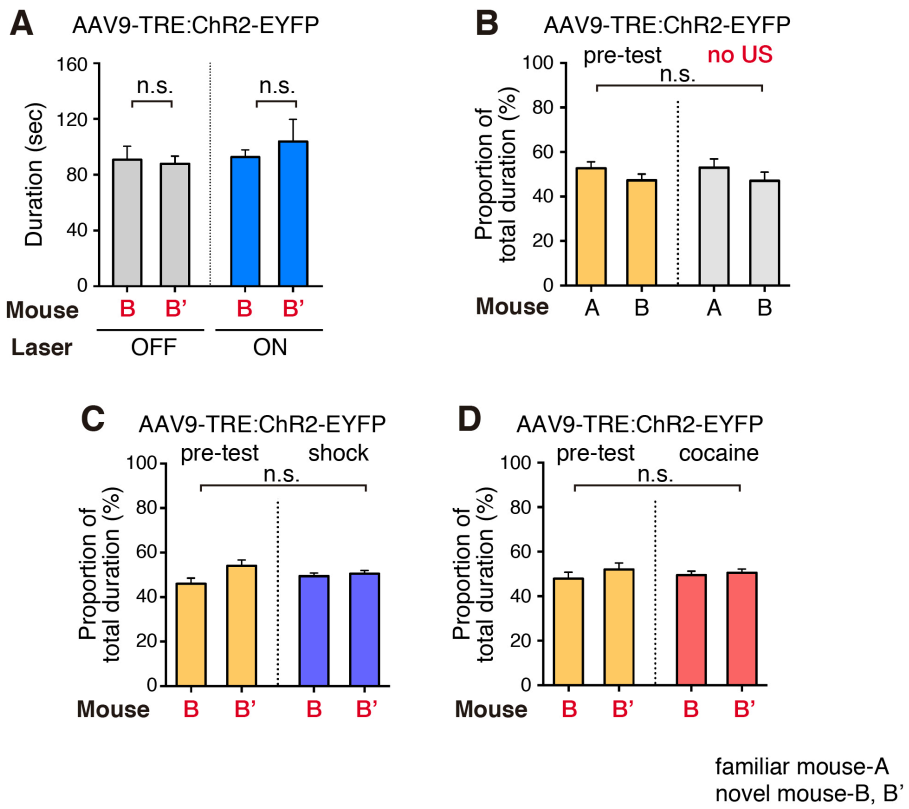

Fig. S15. Control groups for social memory engram experiments.

mouse-A is a familiar mouse, while mouse-B and -B' are novel mice. Total sniffing duration (**A**) or proportion of total sniffing duration (**B** to **D**) in SDT, observed in c-fos:tTA mice bilaterally injected with AAV9-TRE:ChR2-EYFP and implanted with optic fibers targeting vCA1. Social interaction-activated vCA1 neurons (mouse-A neurons) were labeled with ChR2 using the same procedure as Fig. 4, F and K. (**A**) Two different novel mice -B and -B' (instead of familiar mouse-A and novel mouse-B) were used for the optogenetic recall experiments. Light activation did not affect behavior when test-mice interacted with two novel mice. Blue bars, blue laser-ON; Grey bars, laser-OFF (n = 7). (**B**) For memory inception experiments, no unconditioned stimuli (US) group (n = 7). During light stimulation, mice did not receive foot-shocks or cocaine. Light activation of mouse-A neurons without giving any US did not affect test-mice behavior. (**C**, **D**) Two different novel mice -B and -B' (instead of familiar mouse-A and novel mouse-B) were used for the memory inception experiments. Shock-test (**C**, n = 7); cocaine-test (**D**, n = 7). Fear or reward association with mouse-A memory did not affect behavior when test-mice interacted with two novel mice. Yellow bar, pre-test; blue bar, shock-test; red bar, cocaine-test; grey bar, no US-test. Significance for multiple comparisons: paired t-test (**A**) and ANOVA (**B** to **D**); n.s., not significant. Data are presented as mean ± S.E.M.

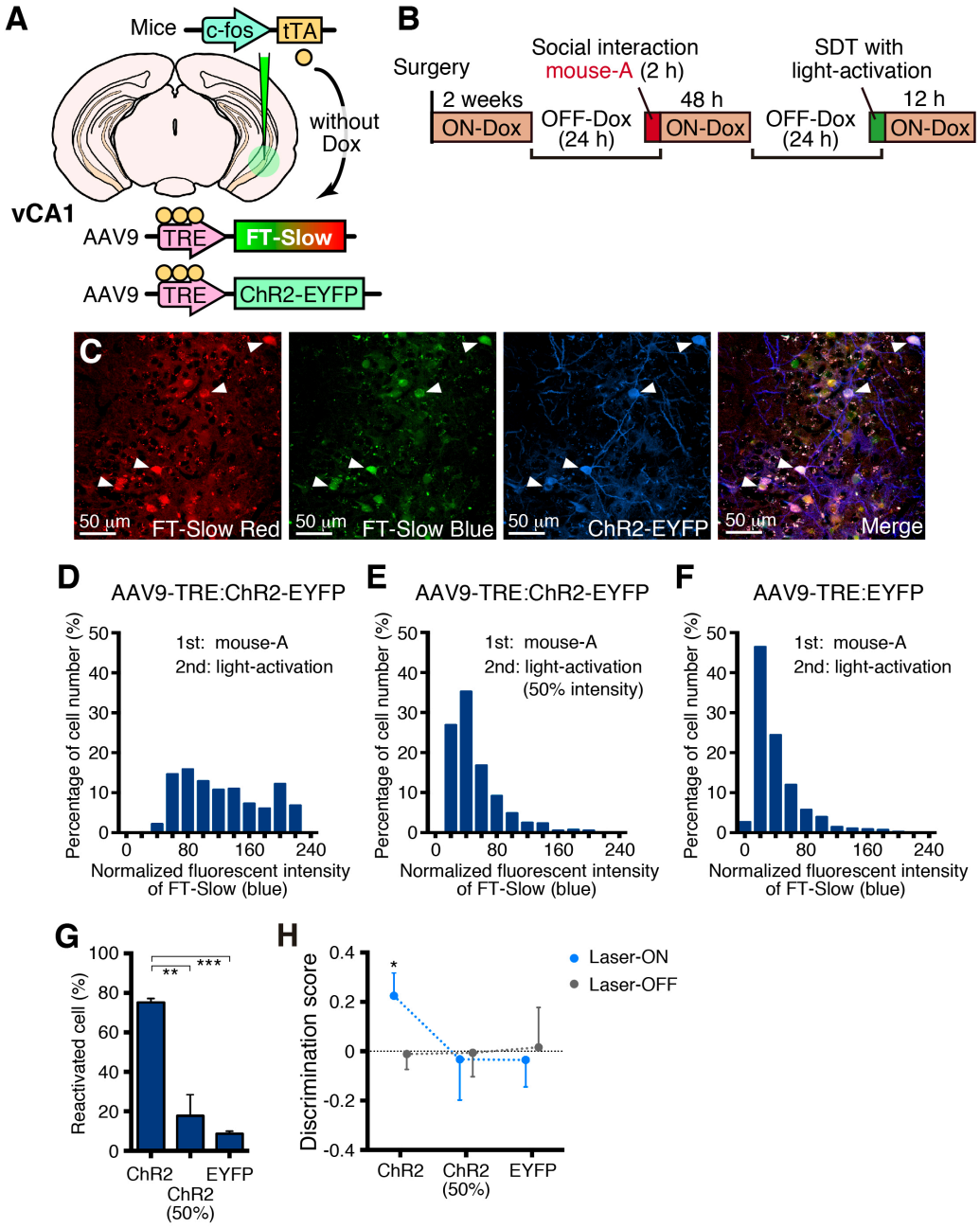

Fig. S16 ChR2-induced reactivation of mouse-A neurons
(**A**) Activity-dependent labeling with FT-Slow and ChR2. Injection of AAV9-TRE:FT-Slow and AAV9-TRE:ChR2-EYFP into vCA1 of c-fos:tTA mice. (**B**) Protocol for visualizing ChR2-induced reactivation of mouse-A neurons. (**C**) Coronal vCA1 sections. FT-Slow blue form (green)-, red form (red)-, and ChR2 (blue)- positive cells. White arrowheads indicate triple merged neurons. (**D** to **F**) Histograms of normalized fluorescence intensity of FT-Slow (blue form) among FT-Slow (red form) positive cells. (**D**) AAV9-TRE:ChR2-EYFP injected group (12 mW), (**E**) AAV9-TRE:ChR2-EYFP injected group (50% laser intensity, 6 mW), and (**F**) AAV9-TRE:EYFP injected group

(12 mW) (n = 3 mice per group). (**G**) Percentage of reactivated cells. (**H**) Comparison of discrimination scores (ChR2, n = 10; ChR2 with 50% laser intensity, n = 6; EYFP, n = 6). Significance for multiple comparisons: ANOVA, post-hoc Scheffe (**G**) and t-test (**H**); *p < 0.05, **p < 0.01, ***p < 0.01, n.s., not significant. Data are presented as mean ± S.E.M.

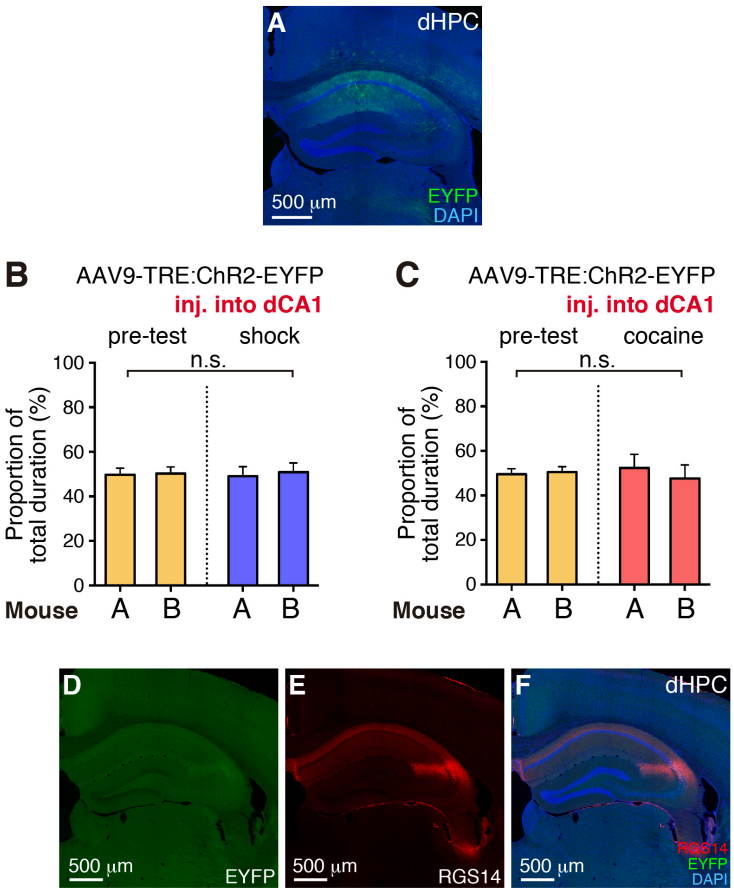

Fig. S17. Social interaction-activated neurons in dCA1 could not support memory inception behaviors.

Same experimental procedure as Fig. 4K was performed using dCA1-targeted mice. (**A**) Coronal dHPC section of a c-fos:tTA mouse injected with AAV9-TRE:ChR2-EYFP into dCA1. Sections were stained with anti-GFP (green) and DAPI (blue). (**B**, **C**) Proportion of total sniffing duration around mouse-A or mouse-B in SDT, observed in c-fos:tTA mice bilaterally injected with AAV9-TRE:ChR2-EYFP and implanted with optic fibers targeting dCA1. Artificial association with foot-shocks (**B**, n = 7) or cocaine administration (**C**, n = 7). Yellow bar, pre-test; blue bar, shock-test; red bar, cocaine-test. Significance for multiple comparisons: ANOVA; n.s., not significant. Data presented as mean ± S.E.M. (**D** to **F**) Coronal dHPC section of a c-fos:tTA mouse injected with AAV9-TRE:ChR2-EYFP into vCA1. Sections were stained with anti-GFP (green), anti-RGS14 (red), and DAPI (blue). EYFP (**D**), RGS14 (**E**), and merged image (**F**).

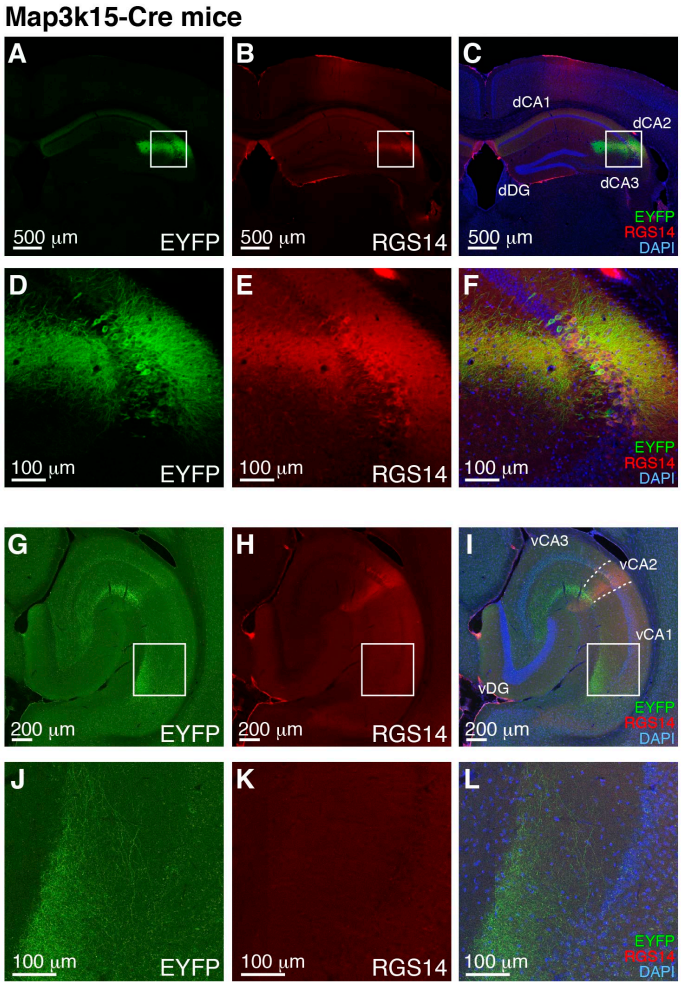

Fig. S18. Dorsal CA2 projections to vCA1.

Coronal dHPC and horizontal vHPC sections of a Map3k15-Cre mouse injected with AAV9-hsyn:DIO-eArchT-EYFP into dorsal CA2 (dCA2). Sections were stained with anti-GFP (green), anti-RGS14 (red), and DAPI (blue). EYFP (**A**, **D**, **G**, **J**), RGS14 (**B**, **E**, **H**, **K**), and merged images (**C**, **F**, **I**, **L**). dHPC images (**A** to **F**) and vHPC images (**G** to **L**). White boxed areas (**A** to **C**, **G** to **I**) are magnified in (**D** to **F**, **J** to **L**).

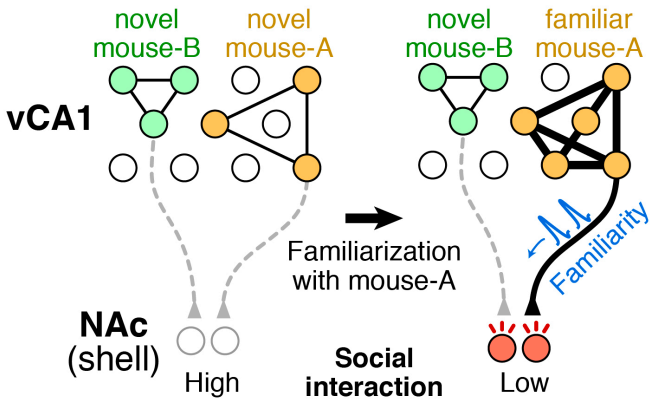

Fig. S19. Model for vCA1-NAc shell circuits underlying social memory and social familiarity.