**Title: A direct lateral entorhinal cortex to hippocampal CA2 circuit conveys social information required for social memory**

**Authors:** Jeffrey Lopez-Rojas[a*], Christopher A. de Solis[a], Felix Leroy[a,b], Eric R. Kandel[a,c] and Steven A. Siegelbaum[a*]

[a]Department of Neuroscience, The Kavli Institute for Brain Science, Mortimer B. Zuckerman Mind Brain Behavior Institute, Vagelos College of Physicians and Surgeons, Columbia University, New York, NY, 10027 USA.

[b]Cellular and Systems Neurobiology Research Unit, Instituto de Neurociencias de Alicante, Alicante, Spain.

[c]Howard Hughes Medical Institute, Columbia University, New York, NY, USA

[*]Corresponding authors. E-mail: jl5545@columbia.edu, sas8@columbia.edu

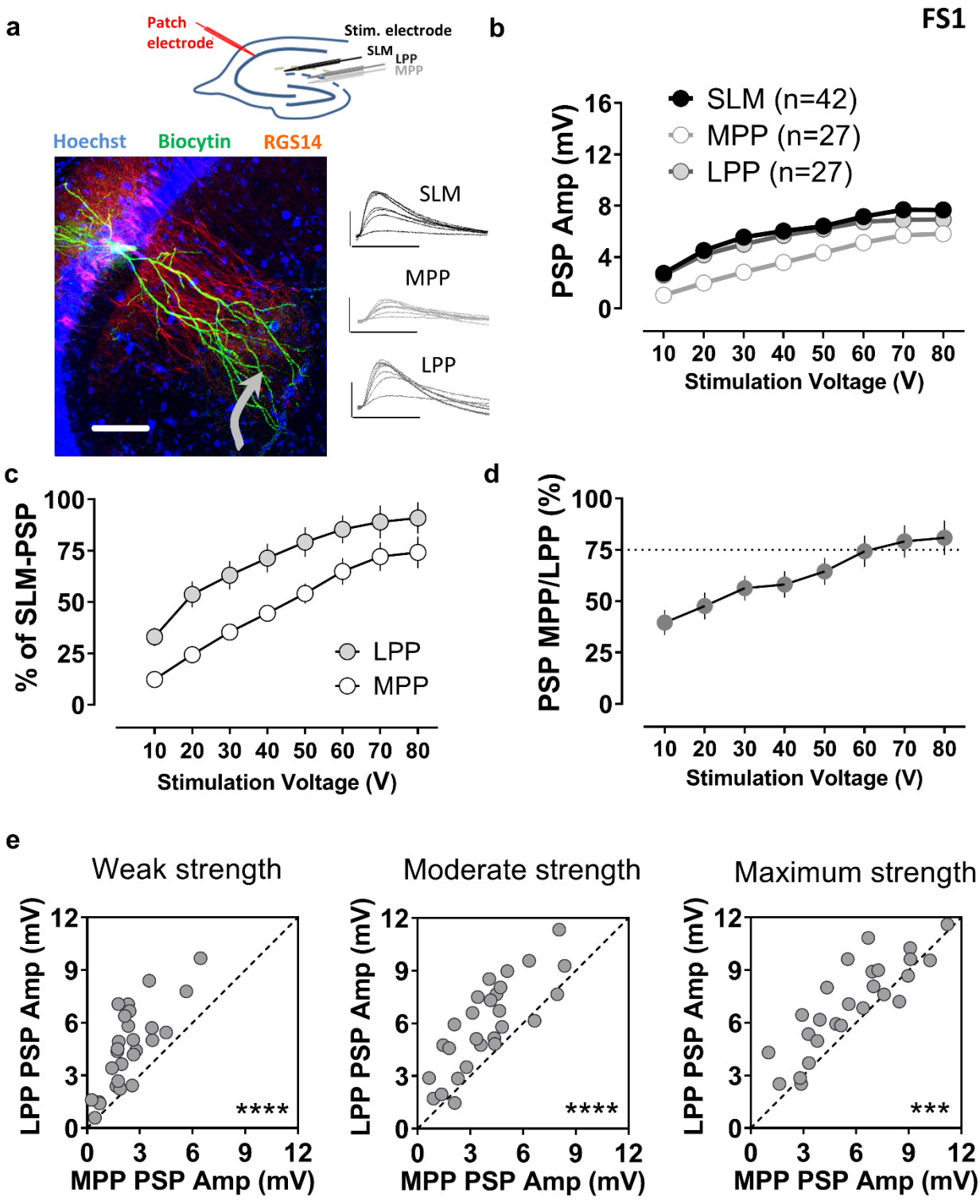


**Figure S1. Electrical stimulation of the lateral perforant path evokes a larger intracellular synaptic depolarization of dorsal CA2 pyramidal neurons compared to stimulation of medial perforant path, Related to Figure 1. a,** Schematic showing the placement of a stimulating electrode (black) in the stratum lacunosum moleculare (SLM) to

globally activate entorhinal axons or in the outer (LPP) or middle (MPP) molecular layer of the dentate gyrus to selectively stimulate lateral or medial perforant path (LPP or MPP). Synaptic responses were measured using patch clamp recordings from CA2 pyramidal neurons. An image of a patch-clamped biocytin-filled CA2 pyramidal cell and the corresponding voltage responses to stimulation in the indicated regions. **b,** Electrical stimulation at any location evoked a large postsynaptic potential in CA2 pyramidal neurons. The LPP-evoked response (27 cells from 27 slices from 14 animals) was larger than the MPP-evoked response (27 cells from 27 slices from 14 animals), expressed as percentage of the SLM-evoked response (42 cells from 42 slices from 15 animals) (**c**), as a ration of the MPP/LPP response (**d**) or as voltage amplitudes (**e**) over a range of stimulation strengths. Scale bars: **a**: 100 µm and 5 mV/25 ms. ****: $p<0.0001$, ***: $p<0.0001$ paired t-test.

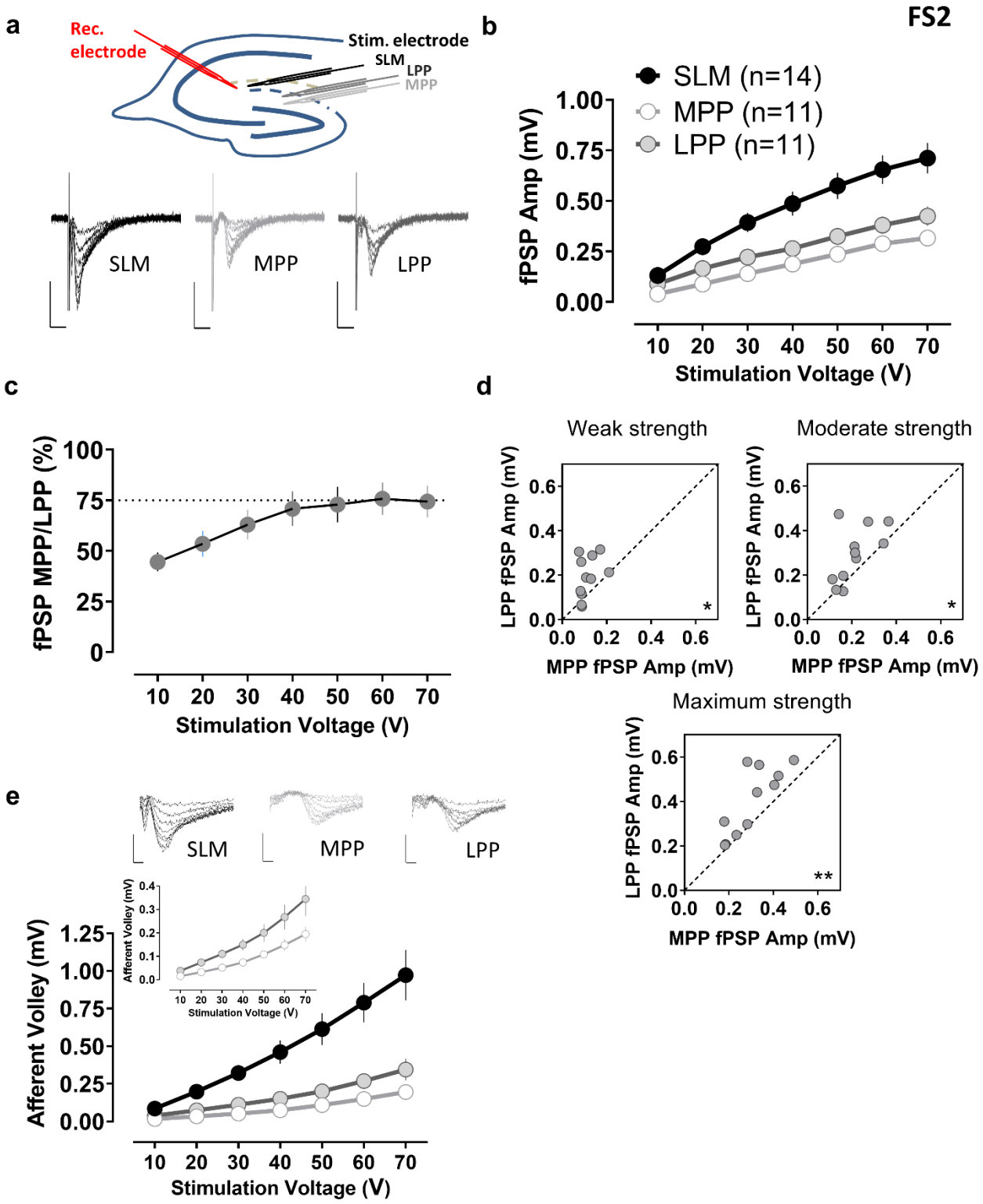


**Figure S2. Electrical stimulation of the lateral perforant path evokes a larger extracellular field potential in SLM of dorsal CA2 region compared to stimulation of**

**medial perforant path, Related to Figure 1. a,** Schematic showing the placement of stimulating electrodes as in Figure S1, while extracellularly recording from CA2 distal dendrites in SLM. The insets show representative field potentials. **b,** Electrical focal stimulation in SLM evoked a large field potential in CA2 distal dendrites (14 slices from 6 animals). The LPP-evoked response (11 slices from 5 animals) was significantly larger than the MPP-evoked response (11 slices from 5 animals) at all stimulation strengths (**c, d**). **e,** The extracellular presynaptic fiber volley in response to a stimulating pulse, which reflects the number of activated axons, was also larger in LPP than in MPP, suggesting a larger number of synaptic contacts. Scale bars: **a**: 0.5 mV/5 ms, **e**: 0.5 mV/1 ms. *: $p<0.05$, **: $p<0.01$ paired t-test.

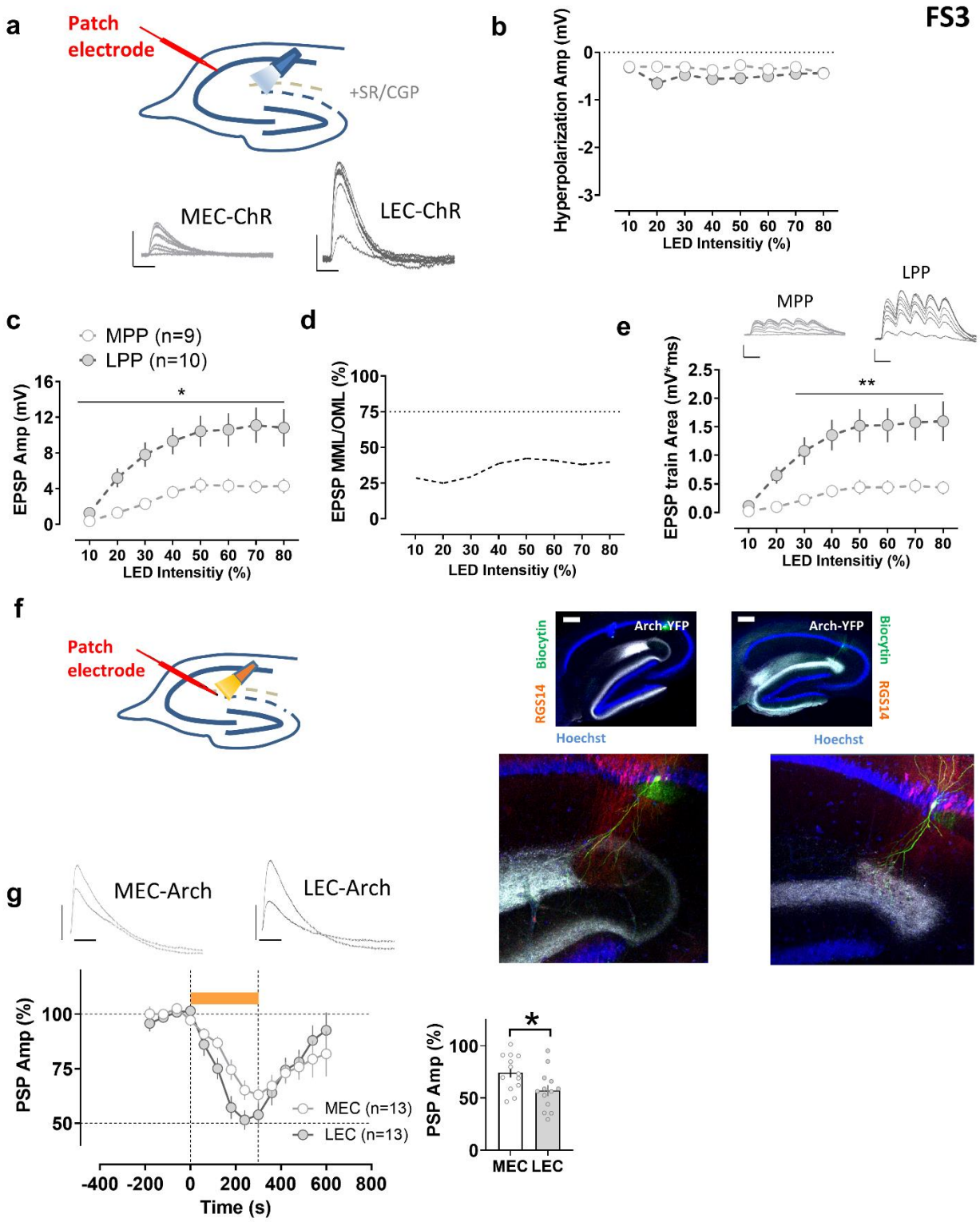


**Figure S3. Optogenetic activation of lateral entorhinal cortex evokes larger excitatory postsynaptic potentials in CA2 pyramidal cells compared to activation of medial**

**entorhinal cortex. Optogenetic inhibition of inputs to CA2 reveals larger contribution of the lateral compared to medial entorhinal cortex, Related to Figures 1, 2 and 3. a,** An AAV was injected to express ChR2 in the medial (MEC) or the lateral entorhinal cortex (LEC). All recordings were done in the presence of GABA receptor blockers to isolate the pure excitatory response (**b**). Pulses of blue light were shone on the stratum lacunosum moleculare while intracellularly recording from CA2 pyramidal neurons. **c, d,** Photostimulation of ChR2-expressing terminals in the stratum lacunosum moleculare evoked a large excitatory postsynaptic potential in CA2 pyramidal neurons for both, MEC (9 cells from 9 slices from 4 animals) and LEC (10 cells from 10 slices from 4 animals) groups. The LEC-evoked response was significantly larger than the MEC-evoked one over a range of stimulation strengths for a single light pulse and for a short train of optical stimuli (**e**). **f,** An AAV was injected in medial (MEC) or lateral entorhinal cortex (LEC) to express Arch. Illumination of the stratum lacunosum moleculare (SLM) with yellow light was used to assess the effect of optogenetic inhibition of LEC (13 cells from 13 slices from 5 animals) or MEC (13 cells from 13 slices from 6 animals) inputs on the postsynaptic depolarization in CA2 pyramidal cells evoked by electrical stimulation using an electrode in SLM. **g,** Temporal course of evoked responses. Yellow light was on for 300 s as shown in the graph. The inset shows recorded responses after 180 s of continuos illumination. Scale bars: 5 mV/25 ms and 200 µm. *: $p<0.05$, **: $p<0.01$ Holm-Sidak's post hoc test after two-way mixed-design ANOVA (in c: $F=5.762$ $p<0.0001$ for interaction Stim LED Intensity x PP M-L; in e: $F=6.885$ $p<0.0001$ for interaction Stim LED Intensity x PP M-L; in g: $F=2.675$ $p=0.0250$ for interaction Time x PP M-L).

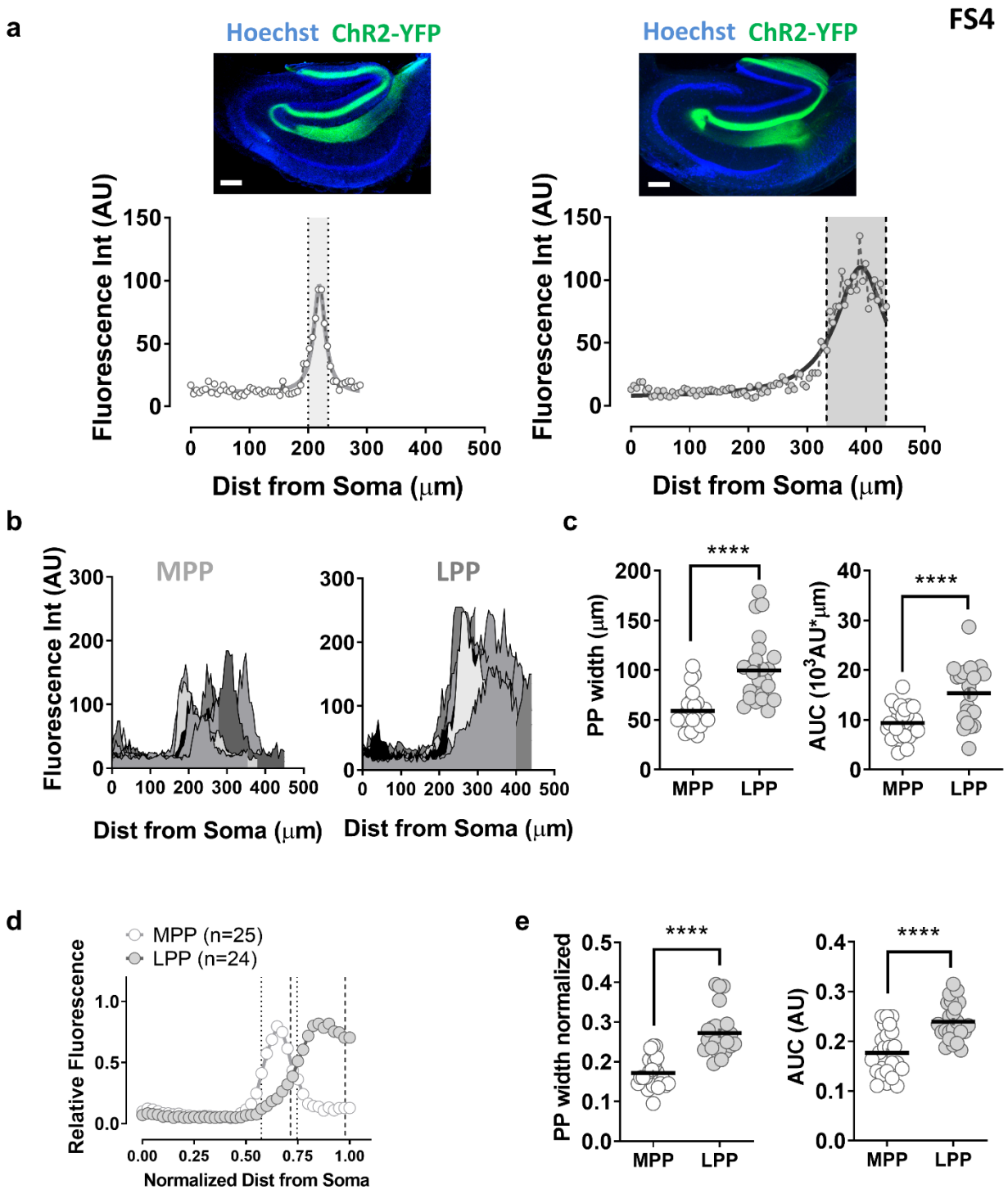


**Figure S4. Lateral entorhinal cortex axons (LPP) occupy a larger area than medial entorhinal cortex ones (MPP) in the stratum lacunosum moleculare (SLM) of CA2, Related to Figure 1. a,** Example images of medial, left, or lateral, right, entorhinal axons in hippocampus labelled by injecting AAV to express ChR2-YFP in MEC or LEC, respectively. Traces below the images show the intensity profiles of entorhinal axon fluorescence for

ChR2-YFP along the CA2 radial axis, from the pyramidal cell layer up to SLM. **b,** Intensity profiles of all analysed slices (MPP: 25 slices from 10 animals, LPP: 24 slices from 10 animals). **c,** LPP occupies a larger area in CA2 SLM than MPP. **d,** Intensity profiles with normalized values of fluorescence and distance. **e**, LPP has a significantly larger width in CA2 SLM than MPP. Scale bar: **a**: 200 µm. ****: $p<0.0001$ t-test.

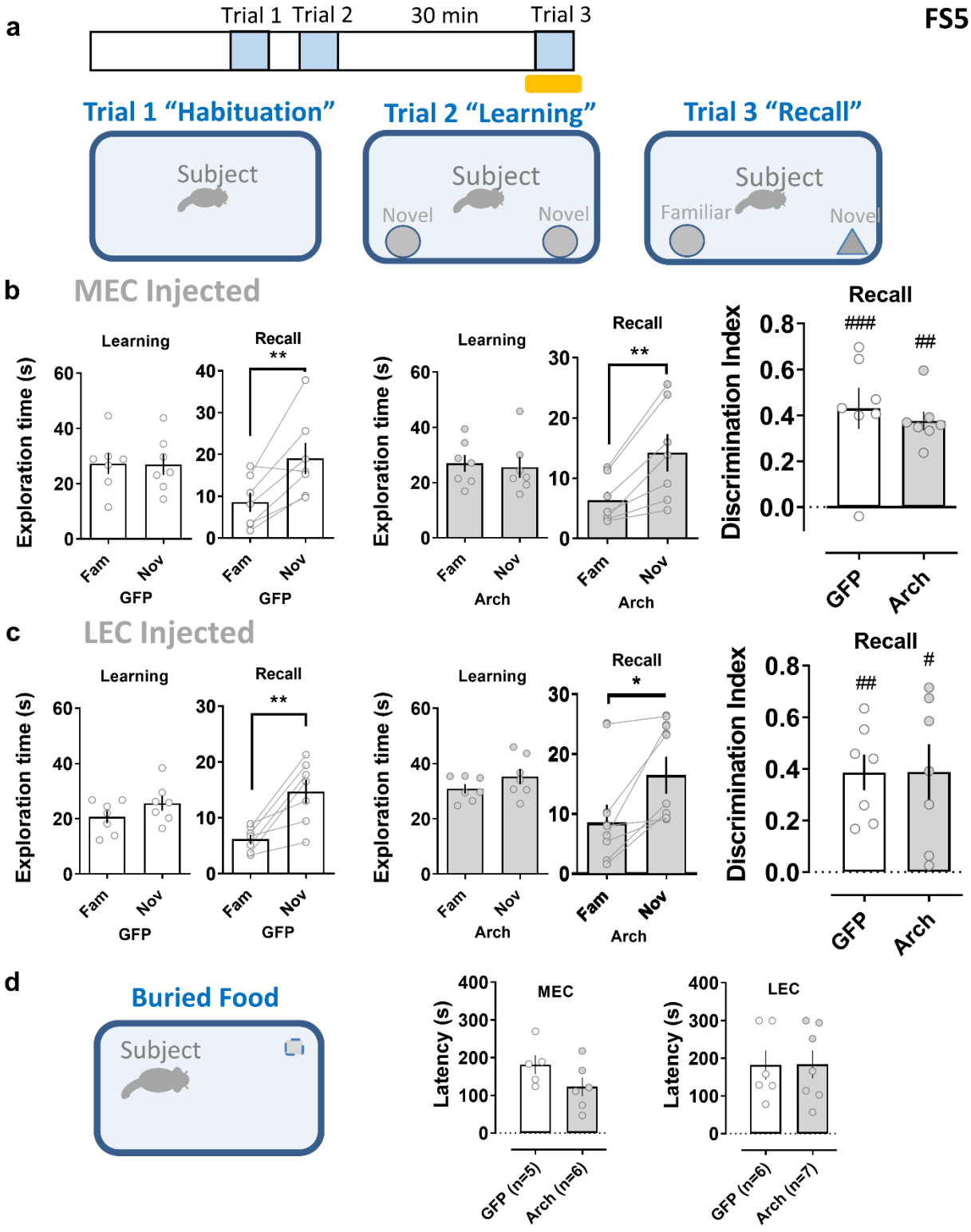


**Figure S5. Disrupting the entorhinal input to dorsal CA2 does not alter novel object recognition or olfactory task performance, Related to Figures 2 and 3. a**, Schema of the novel object recognition task, which is analogous to the two-choice social memory test. Shining yellow light on entorhinal cortex inputs in dorsal CA2 during the recall phase (trial 3)

did not significantly alter the performance of animals previously injected with an Arch-expressing AAV in MEC (7 animals) (**b**) or LEC (7 animals) (**c**), compared to control groups injected with GFP-expressing AAV (GFP MEC: 7 animals, GFP LEC: 7 animals). **c**, Food deprived animals searched to find a buried pellet of food. Shining yellow light on entorhinal cortex inputs in dorsal CA2 did not significantly change the performance of animals previously injected with an Arch-expressing AAV in MEC (6 animals) or LEC (7 animals), compared to GFP-expressing control groups (GFP MEC: 5 animals, GFP LEC: 6 animals). #: $p<0.05$, ##: $p<0.01$, ###: $p<0.001$ one-sample t-test against “0”. *: $p<0.05$, **: $p<0.01$ paired t-test.

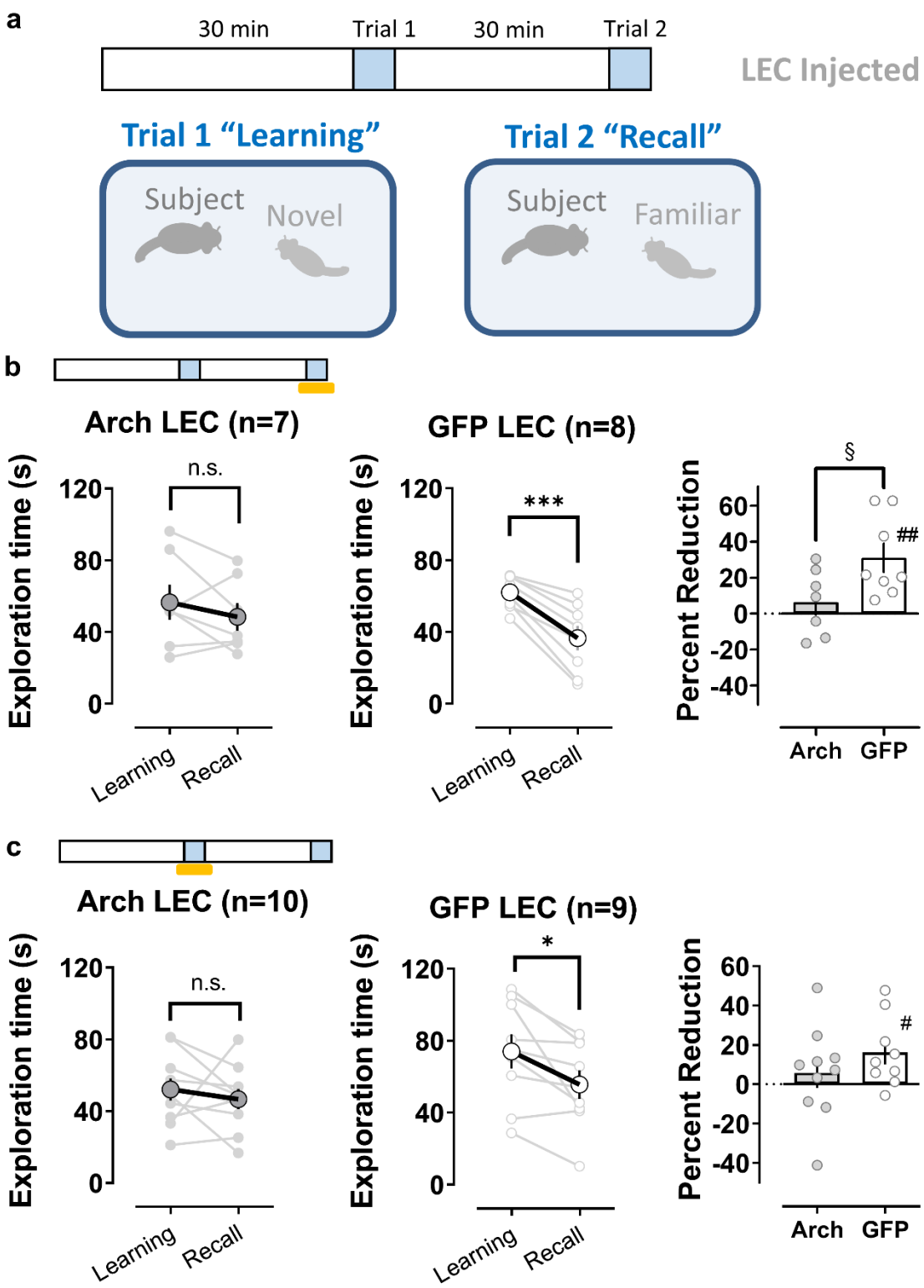


**Figure S6. Disrupting the lateral entorhinal input to dorsal CA2 impairs social memory in the direct interaction task, Related to Figure 2. a**, Schema of the direct interaction

social memory task. A male adult subject mouse was placed in a clean cage for a 30min habituation period. A novel juvenile male mouse was then introduced in the cage and the subject mouse was allowed to explore it for 2 min (trial 1, learning). The juvenile was removed and after a 30 min interval, the same mouse is reintroduced in trial 2. Social memory is manifest as a decrease in exploration of the now familiar juvenile in trial 2 compared to trial 1. Shining yellow light on lateral entorhinal cortex (LEC) inputs in dorsal CA2 either during the recall phase (trial 2, Arch: 7 animals, GFP: 8 animals) (**b**) or during the learning phase (trial 1, Arch: 10 animals, GFP: 9 animals) (**c**), impairs the performance of animals previously injected with an Arch-expressing AAV in LEC compared to the control group expressing GFP in LEC. §: $p<0.05$ t-test. #: $p<0.05$, ##: $p<0.01$ one-sample t-test against “0”. *: $p<0.05$,***: $p<0.001$, paired t-test.

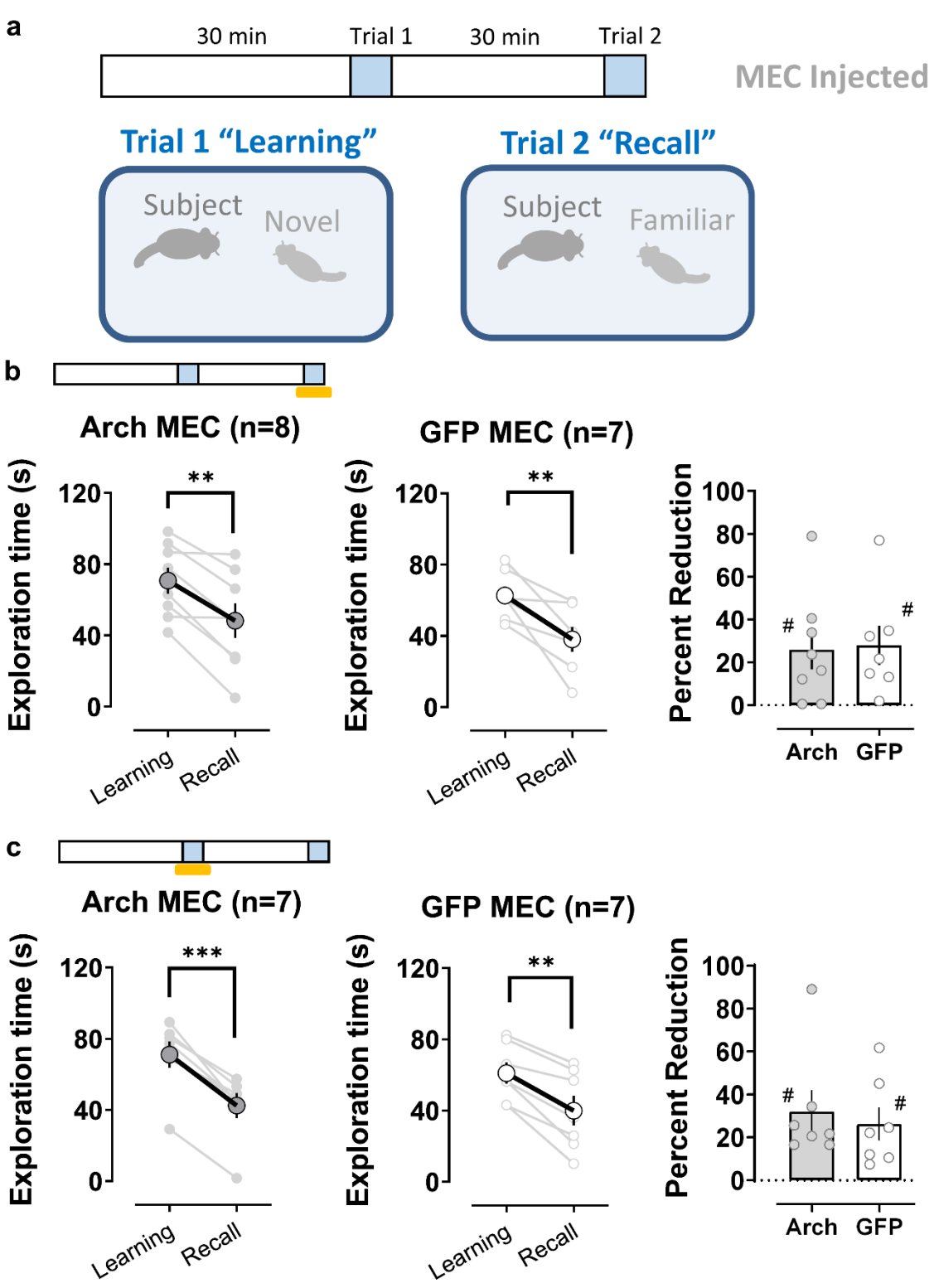


**Figure S7. Disrupting the medial entorhinal input to dorsal CA2 does not influence performance in the direct interaction social memory task, Related to Figure 3. a**, Schema of the direct interaction task consisting of two trials. Shining yellow light on medial

entorhinal cortex (MEC) inputs in dorsal CA2 either during the recall phase of the task (trial 2, Arch: 8 animals, GFP: 7 animals) (**b**) or during the learning phase (trial 1, Arch: 7 animals, GFP: 7 animals) (**c**), does not significantly change the performance of animals previously injected with an Arch-expressing AAV in MEC compared to a control GFP-expressing group. #: $p<0.05$, one-sample t-test against “0”. **: $p<0.01$,***: $p<0.001$, paired t-test.

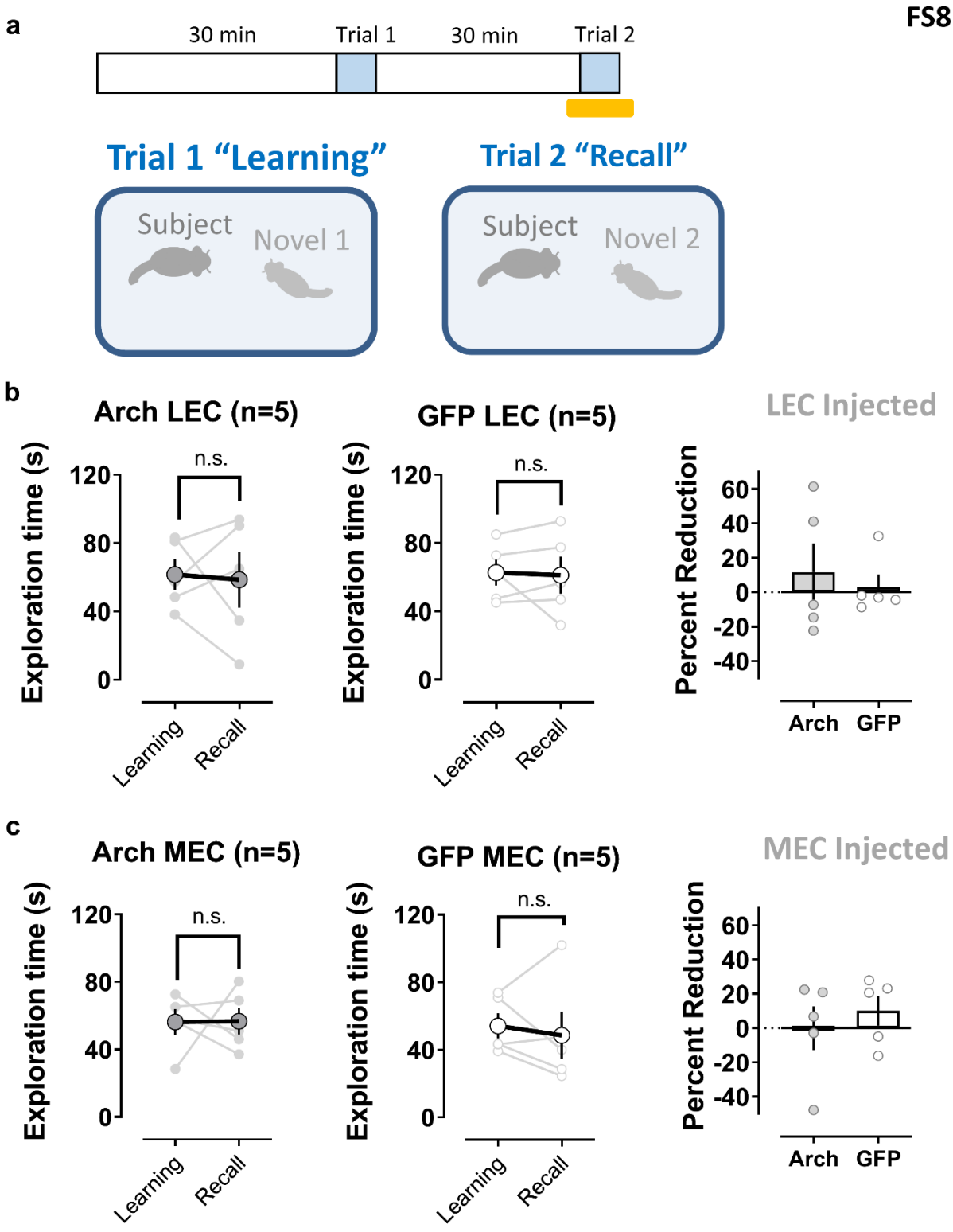


**Figure S8. Decreased exploration time during the recall trial in the direct interaction task does no result from fatigue or lack of motivation for social exploration of the**

**subject mice, Related to Figures 2 and 3. a**, Schema of the behavioral task, a variant of the direct interaction task, consisting of two trials in which a second novel juvenile male was introduced in trial 2. Mice normally show equal exploration of the two novel juveniles, indicating that the decrease in exploration when the same juvenile encountered in trial 1 is reintroduced in trial 2 reflects social memory of the now familiar mouse, rather than fatigue or lack of motivation to explore in the second trial. Shining yellow light on entorhinal cortex inputs in dorsal CA2 during the recall phase (trial 2) did not significantly change the performance of animals previously injected with an Arch-expressing AAV in LEC (5 animals) (**b**) or MEC (5 animals) (**c**), compared to GFP-expressing control groups (GFP LEC: 5 animals, GFP MEC: 5 animals).

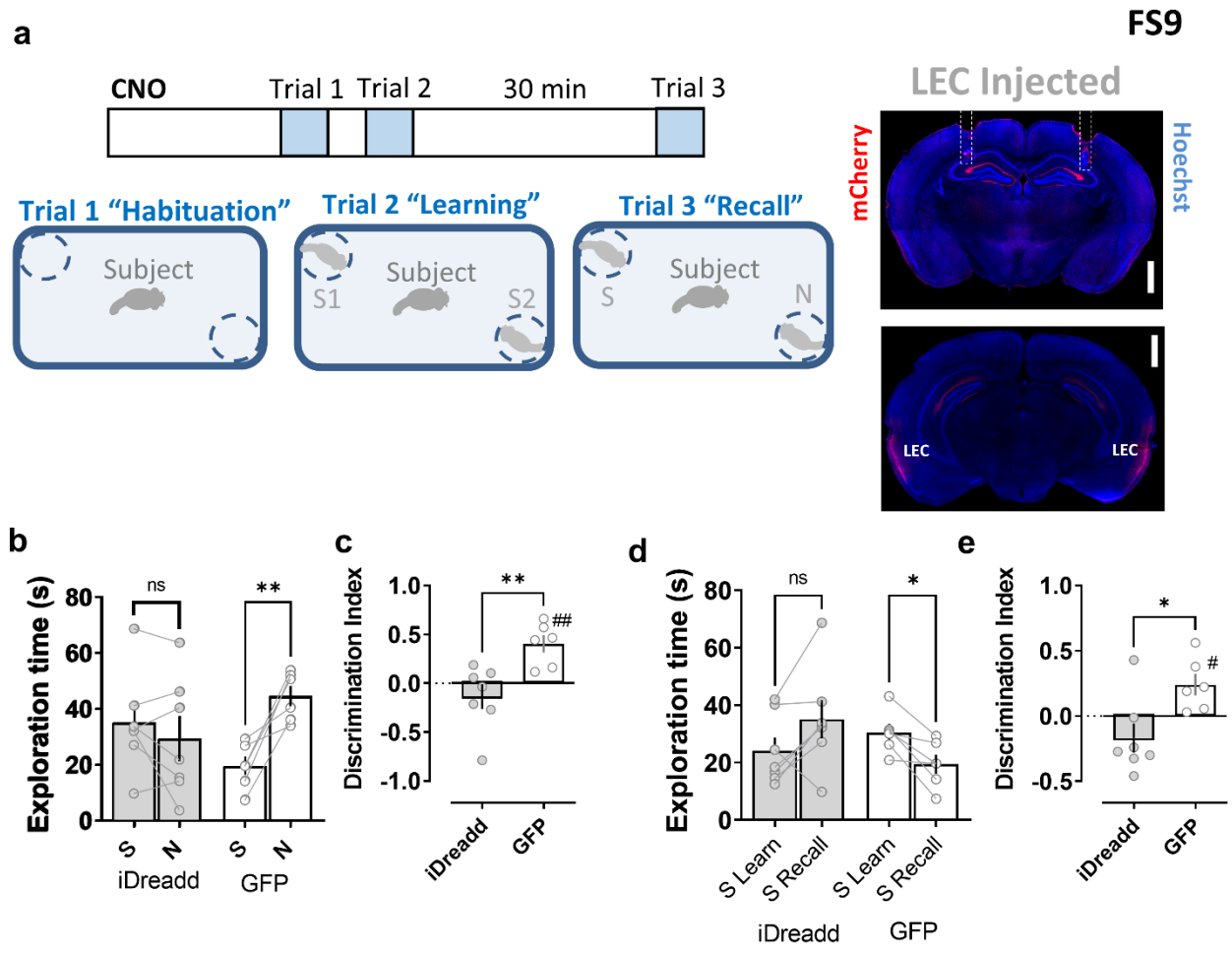


**Figure S9. Pharmacogenetic silencing of the lateral entorhinal cortical input to dorsal CA2 impairs social memory, Related to Figures 2 and 4. a**, Schema of the social memory task described in Figure 2. Insets show the expression of the inhibitory DREADD (iDREADD) hM4Di fused with mCherry in the lateral perforant path and the cannula location (dashed outline) in a coronal brain slice from a mouse previously injected in lateral entorhinal cortex (LEC) with an iDREADD-expressing AAV. **b-e**, Local infusion of the iDREADD agonist CNO (1 mM, 1 µl per side) in dorsal CA2 30 min before the start of the task impairs social memory performance of animals expressing iDREADD in LEC (7 animals) relative to the control group expressing GFP (6 animals). Scale bar: **a**: 1 mm. In **b**: **: p<0.01 Holm-Sidak's post hoc test after two-way mixed-design ANOVA (F=4.912 p=0.0344 for interaction Familiarity x Genotype); in **c**: **: p<0.01 t-test, ##: p<0.01 one-sample t-test against "0"; in **d**: *: p<0.05 Holm-Sidak's post hoc test after two-way mixed-design ANOVA (F=6.778 p=0.0200 for

interaction Familiar L-R x Genotype); in **e**: *: $p<0.05$ t-test, #: $p<0.05$ one-sample t-test against “0”.

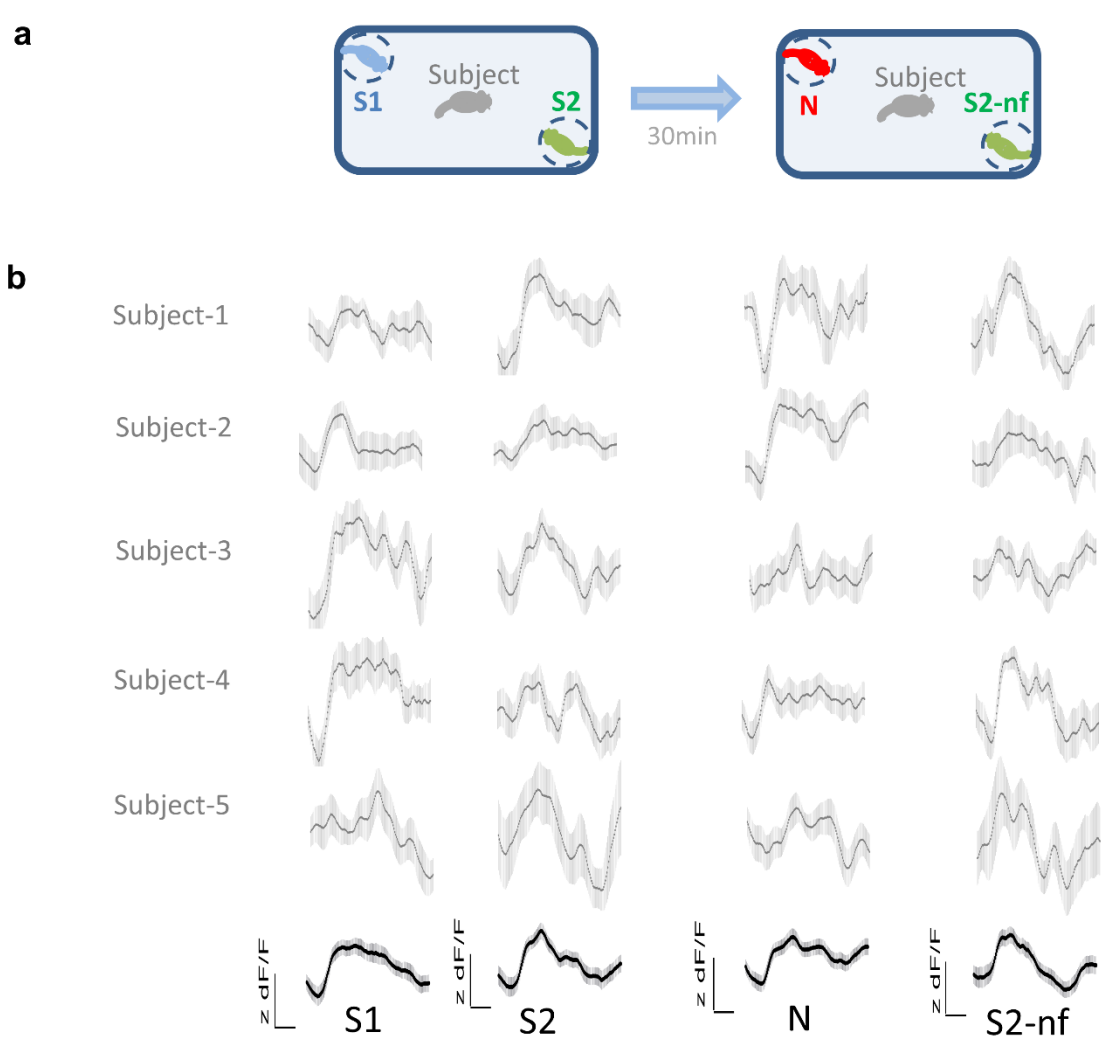


**Figure S10. LEC $Ca^{2+}$ signals from individual mice during two-choice social memory task, Related to Figure 7a-c. a**, Subject mice were allowed to explore the arena with two novel mice (S1 and S2) for 5 min. The subject mouse was then removed from the arena for a 30 min intertrial interval, after which it was reintroduced to the arena and allowed to explore for another 5 min, in which one of the stimulus mice presented in learning trial (eg, S1) was replaced by a third novel mouse (N). **b**, z-scored dF/F traces from single subject animals aligned to the time of interaction. Gray traces show average fluorescence from all interaction bouts of a given type for that subject animal. Black traces show average of all animals (n=5), shown in Figure 7a.

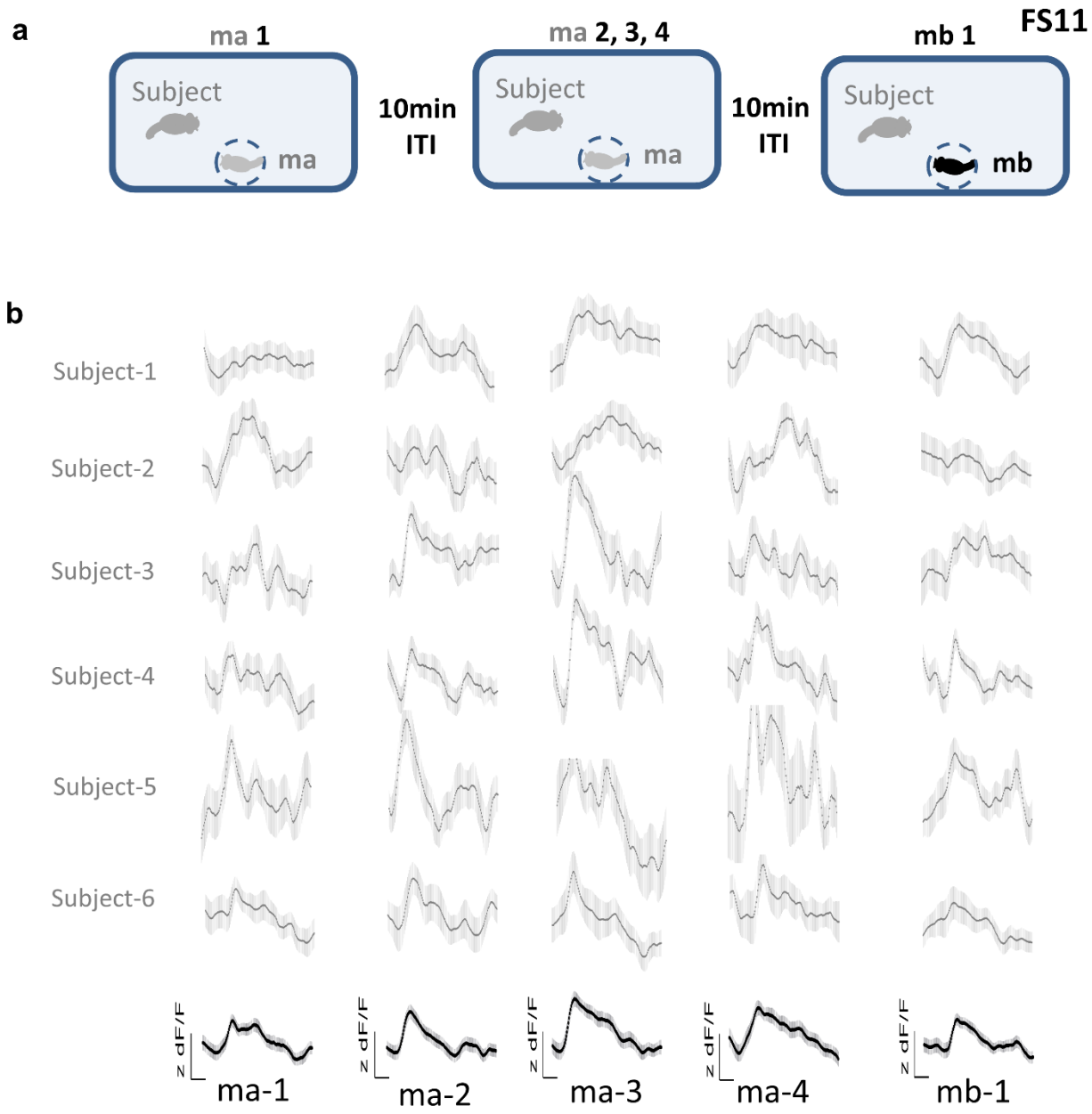


**Figure S11. LEC $Ca^{2+}$ signals from individual mice during five-trial social memory task, Related to Figure 7d-f. a**, Five-trial social memory assay. **b**, z-scored dF/F traces from single subject animals aligned to the time of interaction. Gray traces show average fluorescence signals from all interaction bouts of a given type for that subject animal. Black traces show average of all animals (n=6), shown in Figure 7d.

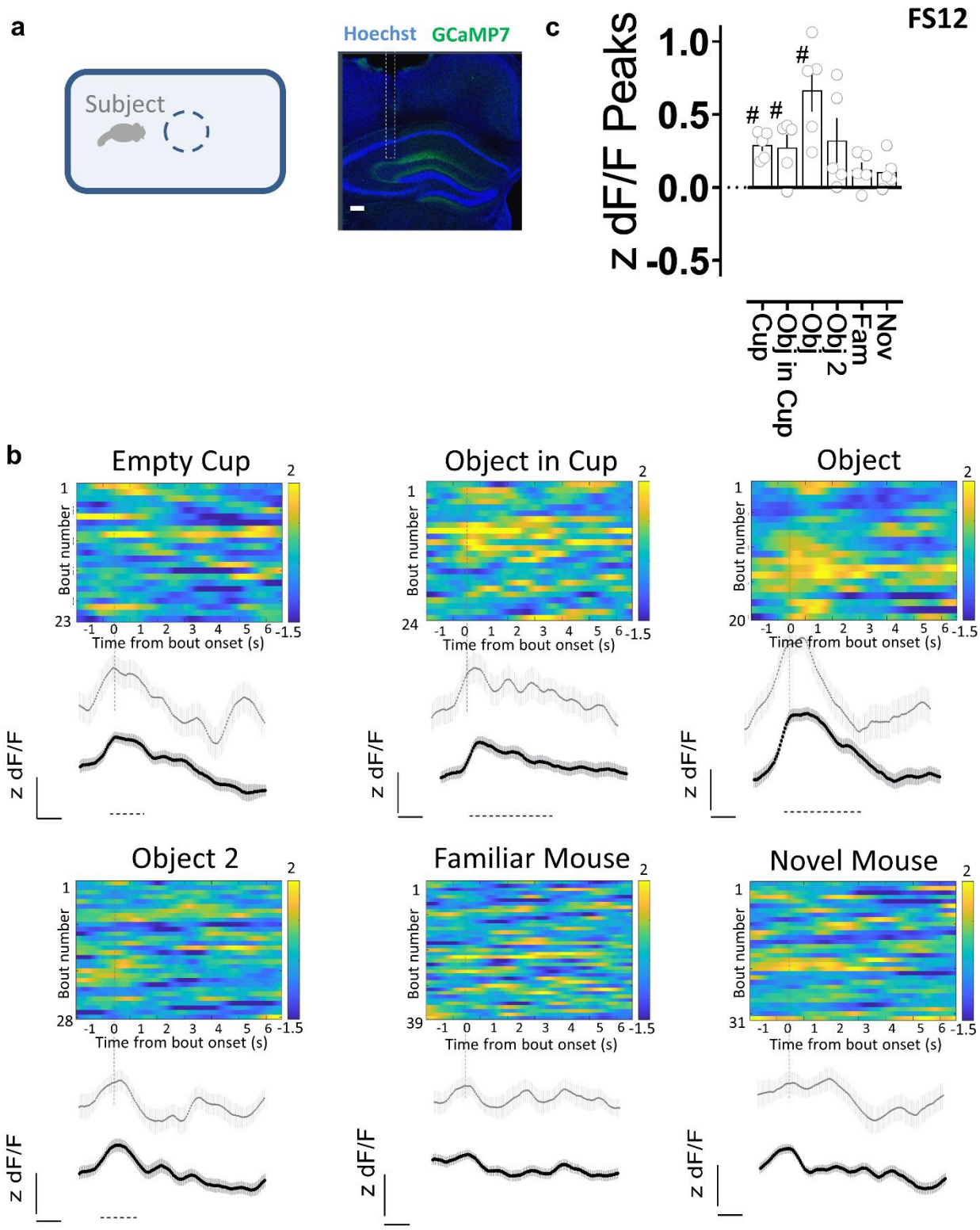


**Figure S12. Activity of the medial entorhinal cortex input to dorsal CA2 does not increase during bouts of social exploration, Related to Figure 6. a**, Fiber photometry

recordings of GCaMP7f fluorescence in medial entorhinal cortex (MEC) inputs to CA2 in dorsal hippocampus while an animal explores indicated items in an open arena. Coronal section of the hippocampus showing the expression of GCaMP7f and the optical fiber location (dashed outline) in mice previously injected with AAV in MEC. **b**, Ca2+ signals in MEC inputs to CA2 during bouts of social or object exploration. Color-coded z-scored dF/F traces from a single animal aligned to the time of interaction. Gray traces show average fluorescence from all interaction bouts of a given type for that animal. Black traces show average of all animals (n=5). Dashed line below the traces indicates the time window with a significant difference with respect to baseline. **c**, Mean peak Ca2+ signals during indicated interactions relative to baseline. Each symbol from a different animal. #: $p<0.05$ one-sample t-test against “0”.