OLM interneurons differentially modulate CA3 and entorhinal inputs to hippocampal CA1 neurons

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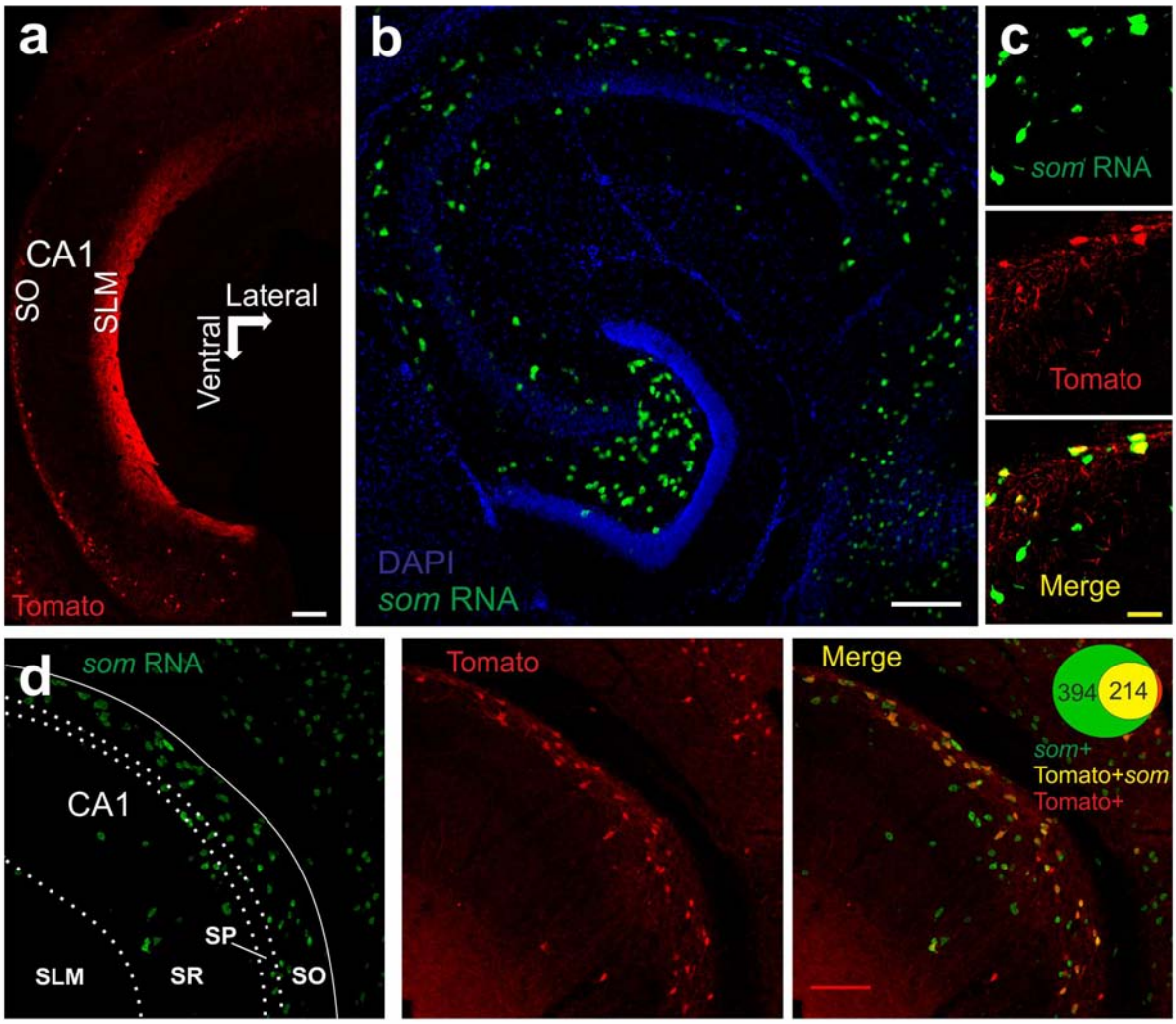
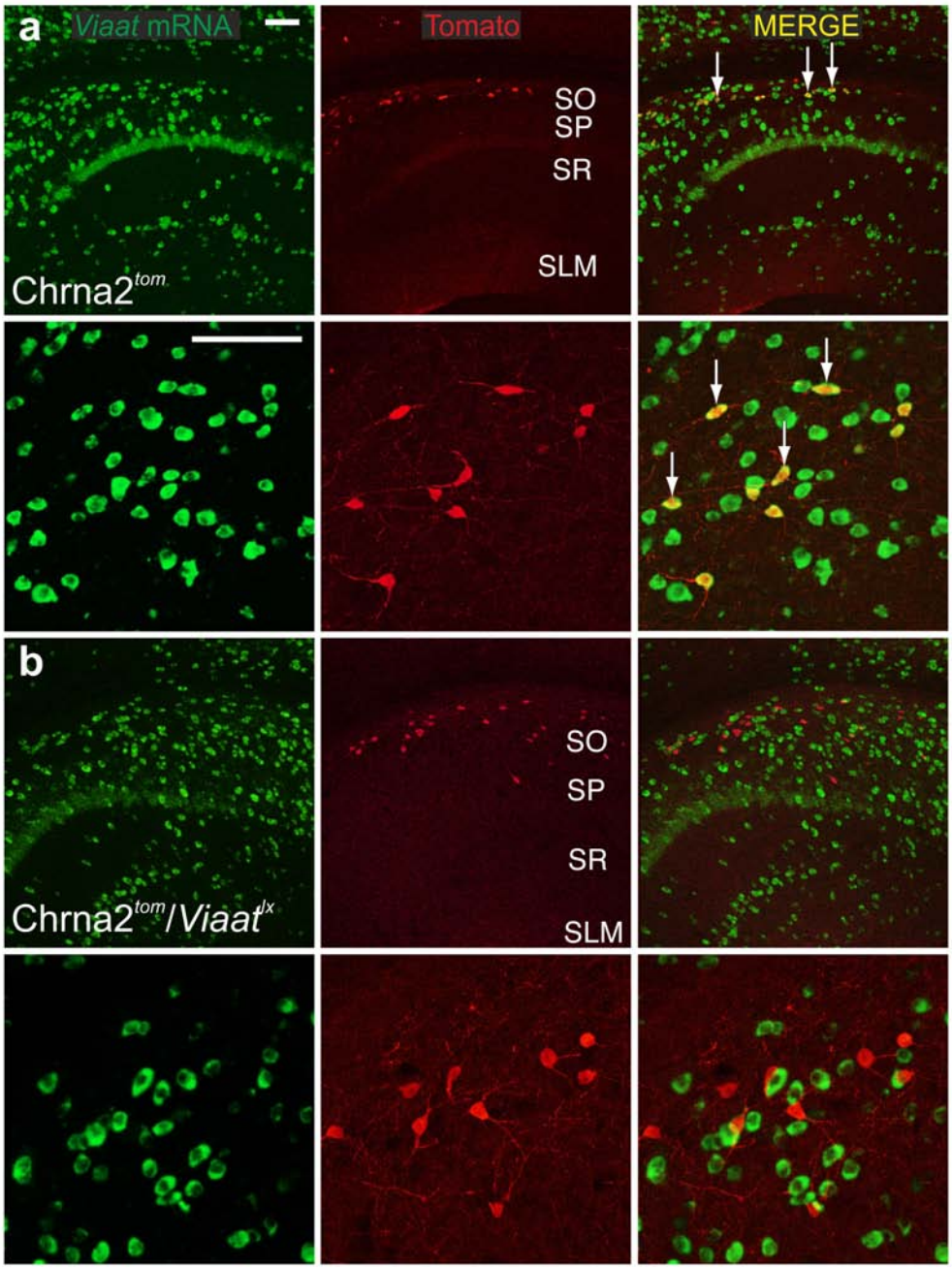
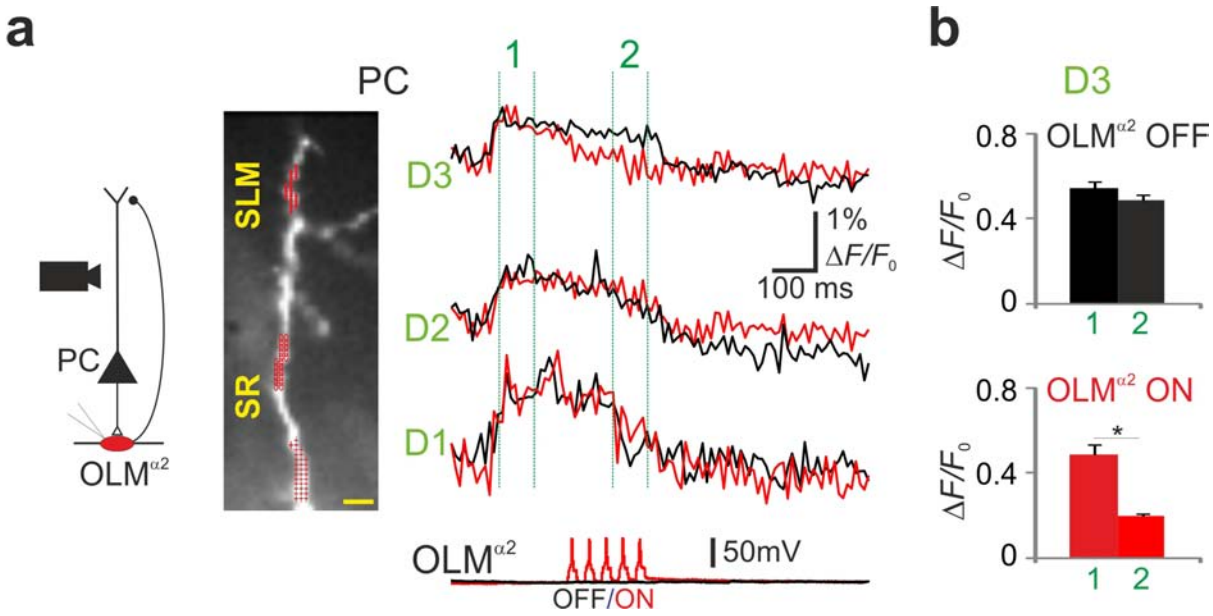
Supplementary Figures 1-9



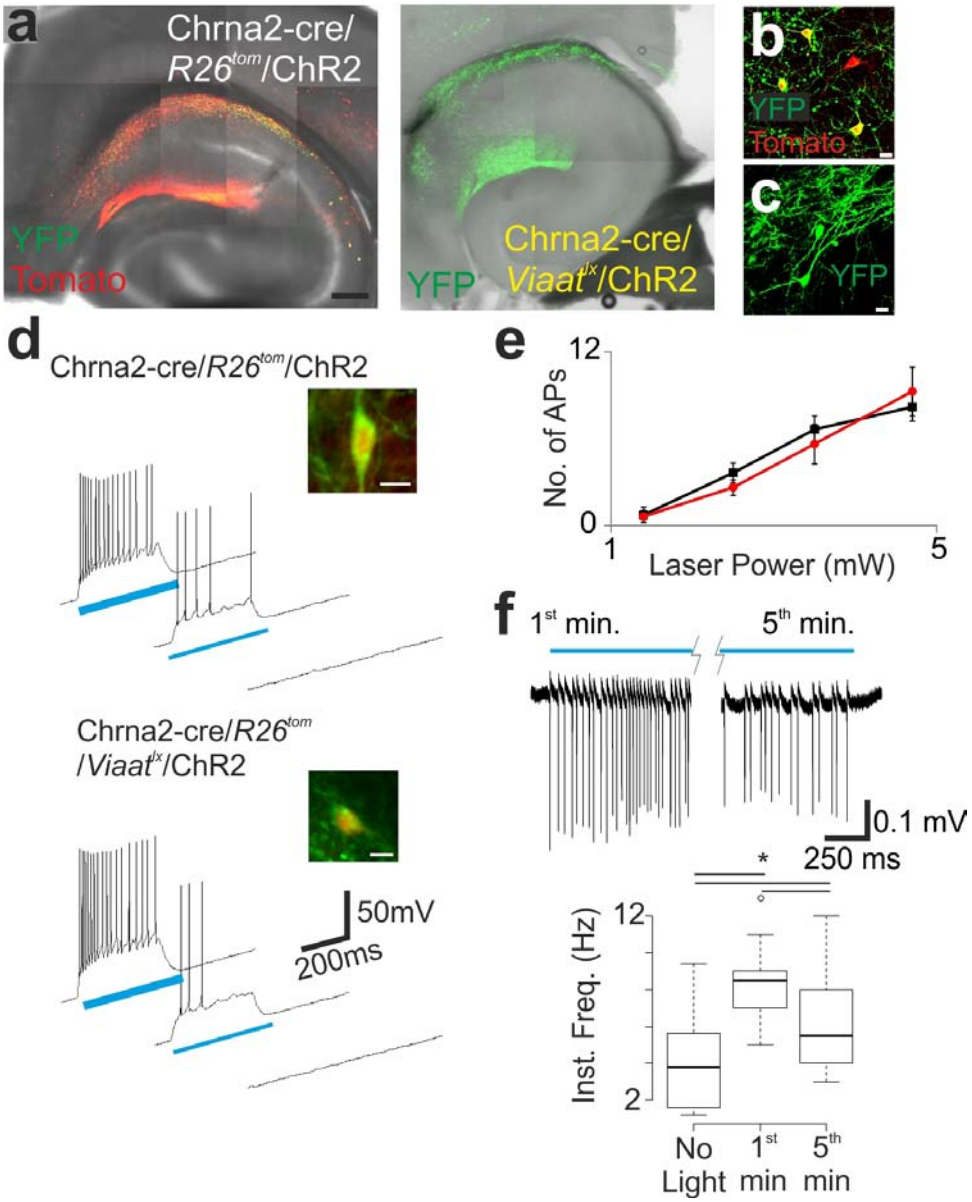
Supplementary Figure 1. Chrna2+ cells are a subpopulation of *som*+ neurons in the SO of CA1 and subiculum. (**a**) Tomato+ cell distribution in fluorescent photomicrographs of a coronal hippocampal section from a Chrna2-cre/$R26^{tom}$ mouse (scale bar=200μm). (**b**) Example of *in situ* hybridization for *som* RNA. (**c**) Examples of coexpression of *som* RNA and the Tomato protein (evinced by immunohistochemistry, scale bar=50μm). (**d**) Another example of *som* and *chrna2* coexpression. *Inset*, Venn diagram showing the number of cells that exclusively expressed *som* (green; n=394), co-expressed *som* and *chrna2* (yellow; n=214) or only *chrna2* RNA (red; n=11).



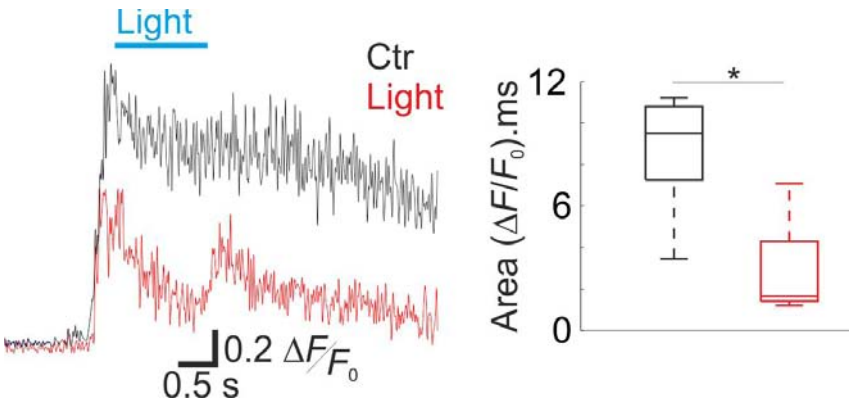
Supplementary Figure 2. *Viaat* mRNA is selectively lost in OLM$^{\alpha 2}$ cells in Chrna2-cre/*Viaat*^{lx} mice. Fluorescent photomicrographs of horizontal hippocampal sections of Chrna2-cre/*R26*^{tom} animals obtained by laser-scanning confocal microscopy. Chrna2-cre activity and *Viaat* mRNA expression in cell bodies were visualized by combined immunofluorescence for Red Fluorescent Protein (RFP, red) to detect tdTomato expressing cells and fluorescent *in situ* hybridization for *Viaat* (green). (**a**) All Chrna2-cre positive cells also expressed *Viaat* mRNA in sections from Chrna2-cre/*R26*^{tom} mice (n=103). Arrowheads indicate cells positive for both *Viaat* mRNA and Chrna2-cre activity. (**b**) The majority (>90%, n=145 out of 160) of Chrna2-cre positive cells in sections from Chrna2-cre/*R26*^{tom}/*Viaat*^{lx} were no longer positive for *Viaat* mRNA expression. SO - stratum oriens, SP - stratum pyramidale, SR - stratum radiatum, SLM - stratum lacunosum moleculare. Scale bar=100µm.



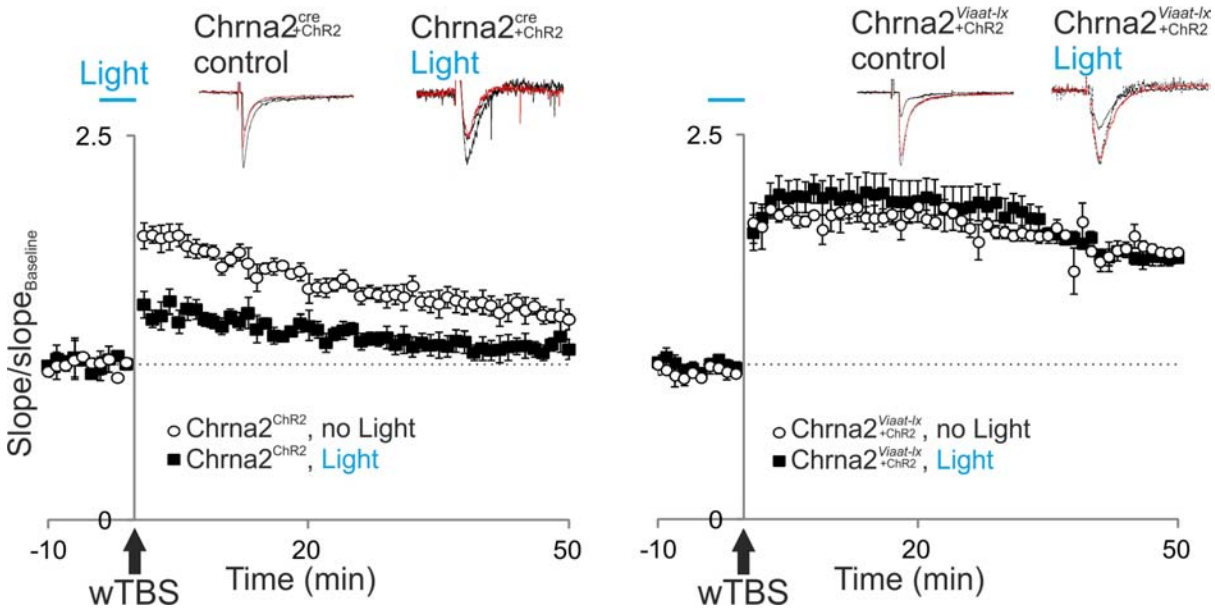
Supplementary Figure 3. OLM$^{\alpha2}$ cells inhibit distal apical dendrites of PCs. (a) Fluorescence changes of a PC apical dendrite filled with voltage sensitive dye (VSD) in response to a depolarizing current step at the PC soma. Recordings were made while a connected OLM$^{\alpha2}$ cell was either silent (black traces, OLM$^{\alpha2}$ OFF) or firing (red traces, OLM$^{\alpha2}$ ON). The current clamp traces of the OLM$^{\alpha2}$ neuron are shown in the bottom. The three sets of fluorescence traces were obtained from three locations of the PC dendrite (shown in red in the micrograph, scale bar=20μm): proximal (D1), intermediate (D2) and distal (D3). (**b**) Mean fluorescence change of the distal PC dendrite (D3) at the onset (period '1' in **a**) and at the end (period '2') of the somatic depolarization step with (bottom) and without (top) OLM$^{\alpha2}$ cell firing (*p<0.05). OLM$^{\alpha2}$ cell spikes were elicited 200ms after the onset of the depolarization step.



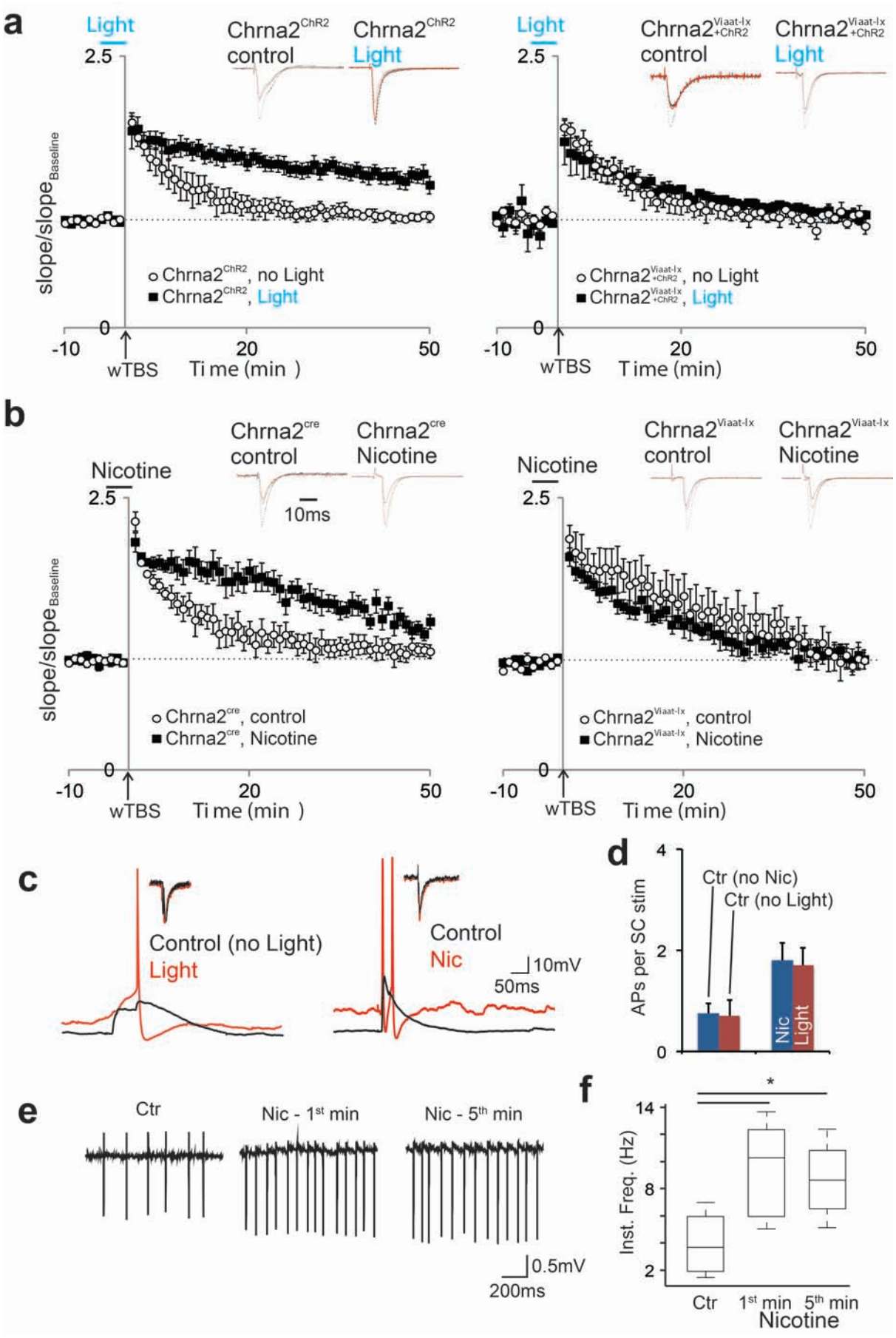
Supplementary Figure 4. Cre driven channelrhodopsin expression is similar in Chrna2-cre/ChR2 and Chrna2-cre/*viaat*^{lx}/ChR2 animals. (**a**) Photomicrograph of a hippocampal section of a Chrna2-cre (left; Tomato reporter) and a Chrna2-cre/*Viaat*^{lx} mouse (right; no reporter) transduced with the light-sensitive channel hChR2 (fused with YFP). Scale bar=200μm. (**b,c**) Expression of YFP in Tomato+ cells in hChR2-YFP-transduced Chrna2-cre/*R26*^{tom} (**b**) and Chrna2-cre/*Viaat*^{lx} animals (**c**). Scale bar=20μm. (**d**) Whole cell responses of hChR2 expressing OLM$^{\alpha 2}$ cells in hippocampal slices from Chrna2-cre/*R26*^{tom} and Chrna2-cre/*R26*^{tom}/*Viaat*^{lx} mice in response to 2.5mW and 3.5mW laser pulse. (**e**) Total laser power vs. number of APs in response to a 400ms laser pulse in OLM^{ChR2} cells in Chrna2-cre (black trace) and Chrna2-cre/*Viaat*^{lx} (red trace). (**f**) Top, cell-attached recordings showing firing adaptation of OLM^{ChR2} cells in Chrna2-cre mice during a 5-min 1.4mW laser pulse. Bottom, boxplot summarizing the firing frequency of OLM^{ChR2} neurons at rest, and during the 1st and 5th minute following the onset of the laser pulse (*p<0.05).



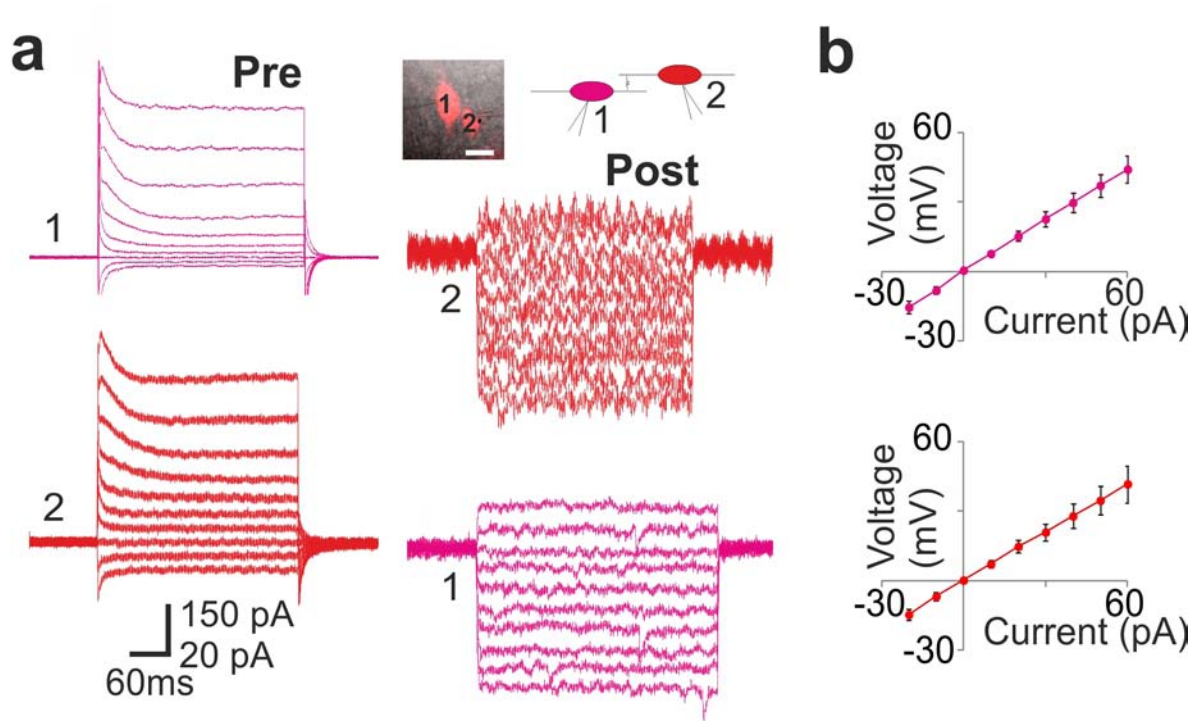
Supplementary Figure 5. OLM cell stimulation inhibits TA excitatory responses at SLM. Fluorescence changes at SLM following TA stimulation in control with or without application of a 1.4mW laser light pulse (left). Excitation of OLM^{ChR2} cells with light produced a large inhibition at SLM measured as the area under the curve of the fluorescence signal change in response to TA stimulation (right). *p<0.05.



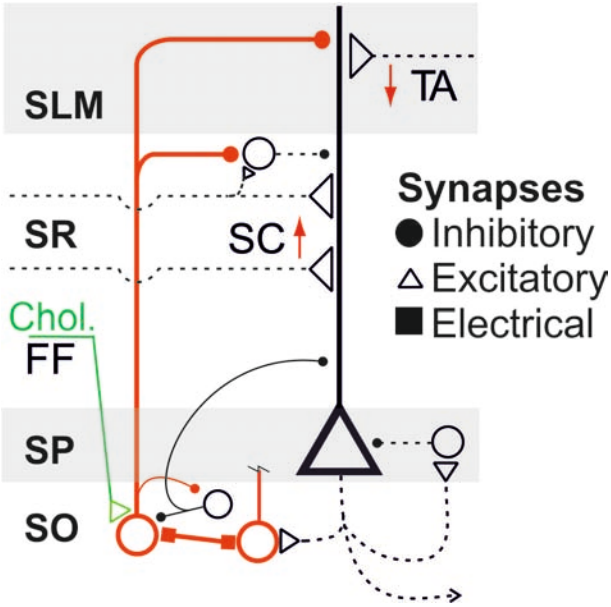
Supplementary Figure 6. Light activation of $OLM^{\alpha 2}$ cells suppresses LTP in the TA pathway. Left, potentiation of TA-PC synapses in Chrna2-cre/ChR2 mice during control conditions (no light; open circles) or upon blue light stimulation (black squares). Right, as before but for Chrna2-cre/*Viaat*^{lx}/ChR2 mice. Top traces show fEPSPs before, 10 and 30 min after wTBS.


a
Light
2.5
Chrna2ChR2 control
Chrna2ChR2 Light
slope/slopeBaseline
Chrna2ChR2, no Light
Chrna2ChR2, Light
0
-10
wTBS
20
50
Time (min)
Light
2.5
Chrna2Viaat-Ix+ChR2 control
Chrna2Viaat-Ix+ChR2 Light
Chrna2Viaat-Ix+ChR2, no Light
Chrna2Viaat-Ix+ChR2, Light
b
Nicotine
2.5
Chrna2cre control
Chrna2cre Nicotine
10ms
Chrna2cre, control
Chrna2cre, Nicotine
Chrna2Viaat-Ix control
Chrna2Viaat-Ix Nicotine
Chrna2Viaat-Ix, control
Chrna2Viaat-Ix, Nicotine
c
Control (no Light)
Light
Control
Nic
10mV
50ms
d
APs per SC stim
Ctr (no Nic)
Ctr (no Light)
Nic
Light
e
Ctr
Nic - 1st min
Nic - 5th min
0.5mV
200ms
f
Inst. Freq. (Hz)
Ctr
1st min
5th min
Nicotine

Supplementary Figure 7. Light activation of OLM$^{\alpha 2}$ cells induces LTP in the SC pathway (**a**) Left, potentiation of SC-CA1 synapses in Chrna2-cre/ChR2 mice during control conditions (no light; open circles) or upon blue light stimulation (black squares). Right, as before but for Chrna2-cre/*Viaat*^{lx}/ChR2 mice. Top traces show fEPSPs before, 10 and 30 min after wTBS. (**b**) Left, potentiation of SC-CA1 synapses in Chrna2-cre mice without (open circles, control) and with application of 1μM nicotine (black squares). *Right,* As before but for Chrna2-cre/*Viaat*^{lx} mice. (**c**) Current clamp recordings showing membrane potential changes in OLM$^{\alpha 2}$ cells following a single SC stimulation in control conditions or in the presence of light or nicotine. (**d**) Mean number of APs triggered in OLM$^{\alpha 2}$ cells by a single SC stimulation in control conditions or in the presence of light or 1μM nicotine. Notice similar changes in resting membrane potential with light and nicotine application. (**e**) Cell attached recordings of OLM$^{\alpha 2}$ neurons in control conditions and in response to the application of 1μM nicotine (example recordings during the 1st and the 5th minute of nicotine application). (**f**) Summary of firing frequency of OLM$^{\alpha 2}$ neurons in control conditions, 1st and 5th minute after nicotine application (* denotes p<0.01).



Supplementary Figure 8. OLM$^{\alpha 2}$ cells are electrically connected. (a) Presynaptic depolarizing voltage steps (-20 to +60mV, -60mV holding) resulted in pre- and postsynaptic outward currents in a CA1 OLM$^{\alpha 2}$ cell pair (photomicrograph on the top, scale bar=20μm). **(b)** Mean junction potential vs. postsynaptic current relationships (bidirectional; 1→2, 2→1) of four electrically connected CA1 OLM$^{\alpha 2}$ cell pairs.



Supplementary Figure 9. Schematic representation of the CA1 OLM cell microcircuit. Red arrows represent the potentiation effect from OLM$^{\alpha 2}$ cell (red) activation on SC-CA1 and TA-CA1 synapses. Cholinergic inputs from the fimbria fornix (FF) are represented in green. Other interneurons represented here are basket cells in SP, SC-associated interneurons in SR and BS neurons in SO (SLM, *s. lacunosum-moleculare*; SR, *s. radiatum*; SP, *s. pyramidale*; SO, *s. oriens*; TA, temporoammonic pathway; SC, Schaffer collateral pathway).